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The restoration of ecological interactions : considering plant-pollinator, host-parasite and alien-alien interactions

THE RESTORATION OF ECOLOGICAL INTERACTIONS:

CONSIDERING PLANT-POLLINATOR, HOST-PARASITE, AND ALIEN-ALIEN INTERACTIONS

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ABSTRACT

1. The studies described here primarily aim to evaluate the potential for ecological restoration to reinstate ecological structure and function to previously degraded communities. In particular, I investigated the extent to which key interactions between species have been re-established at restored sites.

2. The interactions between the plants and pollinators, and between pollinators and their parasites of dry, lowland heathland communities were the main focus of these studies. A food web approach was used to analyse the structural and functional characteristics of plant-pollinator and plant-pollinator-parasite interaction networks, comparing restored and reference heathland communities.

3. With regard to plant-pollinator networks, both the plant and visitor communities were successfully reinstated to restored sites, although the complexity of their interaction networks were reduced relative to reference communities. Despite this, the core pollinator guild was successfully re-established. However when investigating the restoration of the natural enemies of a key pollinator guild, the bumblebees, the establishment of the dipteran parasitoid family Conopidae, remained incomplete. Such higher trophic level specialists can therefore act as potential indicators of restoration success.

4. Further investigation into the patterns and prevalence of the otherwise hidden community of bumblebee natural enemies was undertaken, providing the first comprehensive, quantitative assessment of bumblebee parasitism in the UK. Parasitism was found to be relatively common, with prevalence dependent on factors including host caste, species, and worker size. No conclusive evidence was found for the impact of parasitism on floral species preference or pollen collection by bumblebee hosts.

5. A final investigation assessed the potential for positive interactions between non-native, invasive plant species. With increasing evidence illustrating detrimental synergistic effects of interacting invasive species, the impact of two, co-occurring invasive plant species was studied. However no evidence for facilitation was found when assessing pollinator visitation rates, pollen deposition, pollen limitation, or fruit and seed set.

DEDICATION

Kepada emak saya,

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AUTHOR'S DECLARATION

I declare that the work in this dissertation was carried out in accordance with the Regulations of the University of Bristol. The work is original except where indicated by special reference in the text, and no part of the dissertation has been submitted for any other academic award. Any views expressed in the dissertation are those of the author.

Katherine S.E. Henson

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CONTENTS

CHAPTER ONE

Introduction

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1.1 Introduction

Restoration ecology

"The conservation mind set is one of loss on a relatively short time horizon, whereas the restoration mind set is one of long-term recovery"

(Young, 2000)

Global biodiversity is in severe decline and rates of species extinction today exceed those recorded during mass extinction events in the fossil record (Ricciardi & Rasmussen 1999; Reid 1997). Currently between 5-20% of global species of fish, birds and mammals are threatened with extinction (Pimm *et al.* 1995), whilst the International Union for the Conservation of Nature (IUCN) predict a global loss of 20,000 flowering plant species over the next few decades. Anthropogenic habitat destruction and fragmentation are considered the primary cause of this loss of biodiversity (Ewers & Didham 2006), yet the area of natural habitat lost annually continues at a rate of 0.5%, whilst over 60% of ecosystems remain degraded or are used unsustainably (MEA 2005). Indeed land-use change has been predicted to exert the greatest impacts upon global biodiversity by the year 2100 (Sala *et al.* 2000).

With biodiversity continuing to decline at exponential rates (Raven 2002), and forecasts for global decline in basic ecosystem services (MEA 2005) in spite of numerous conservation strategies and policies identifying and designating protection to critical species and ecosystems, the need for ecological restoration is increasingly recognised (Young 2000; Young *et al.* 2005; Palmer *et al.* 2006). By restoring degraded habitats, ecological restoration has the potential to increase habitat availability and landscape connectivity through the provision of buffer zones and dispersal and recruitment corridors that connect habitat fragments (Hobbs 1992; Bennett *et al.* 1994; Anderson 1995; Murcia 1995; van Dorp *et al.* 1997). By doing so, biodiversity can thus be reinstated to degraded areas where previous conservation management of its remaining species may no longer be adequate or practical (Dobson *et al.* 1997).

The field of restoration ecology has therefore significantly advanced over the last decade. Drawing from ecological theory, restoration ecology focuses on the

amelioration of anthropogenic damage and the reinstatement of self-sustaining ecosystems through the processes of ecological restoration (Cairns & Heckman 1996). It thereby provides a suite of tools that effectively speed up the restoration process necessary for its long-term recovery (Dobson *et al.* 1997).

However deciding upon the goals or 'end-points' of ecological restoration, or determining the specific attributes that define restoration success, are highly problematic (Simberloff 1990; Ehrenfeld 2000; Ruiz-Jaen & Aide 2005). The U.S. National Research Council (NRC) defined ecological restoration as "returning an ecosystem to a close approximation of its condition prior to disturbance [whilst] ensuring that ecosystem structure and function are recreated or repaired, and that natural dynamic ecosystem processes are operating effectively" (NRC 1992). Yet quantitative comparative data, or reference sites equal to the "condition prior to disturbance" are typically limited (Cairns & Heckman 1996; White & Walker 1997), whilst economic and social constraints frequently dictate the longevity of the restoration programme (Handel 1997; Higgs 1997). Consequently, many restoration projects fall short of reinstating adequately functioning systems that are self-regulating and self-sustaining (Pedroli & Harms 2001; Baron *et al.* 2002; Suding *et al.* 2004). In this chapter I introduce the key problems and current directions in ecological restoration, and discuss the importance of restoring ecological interactions to degraded systems. The main aims and structure of this thesis are outlined thereafter, whilst for each of the four data chapters that follow I introduce their specific subject areas in turn.

Determining the goals of restoration

> *"Restoration is a game with a moving target whose trajectory cannot be accurately predicted, and the target in any event cannot quite be seen or characterised"*
>
> (Simberloff 1990)

Determining what to restore, or the resolution to restore to, in the face of ongoing, inherent variation of natural systems over both space and time, presents considerable challenges to restoration ecologists and is a subject of much debate (Aronson & LeFloch 1996; Hobbs & Norton 1996; Palmer *et al.* 1997; Michener 1997; Parker

1997; White & Walker 1997). Indeed the end point of past restoration projects has encompassed both extremes, from restoring a single, target species, to restoring whole landscapes. With regard to the former however, simply the presence of the target species at the restored system is inadequate to guarantee its persistence. This is due to the considerable changes in species composition that occur over short periods of time as a consequence of natural variation (Herrera 1988; Petanidou & Vokou 1993; Fishbein & Venable 1996; Minckley *et al.* 1999; Heywood & Iriondo 2003). Other studies have instead implemented surrogate measures to restore, whereby the presence of a particular group of species (e.g. indicator species, or focal taxa), provides an indication of the presence of essential attributes in the restored system that are required by the remaining biota (Lambeck 1997). Such surrogate measures have also been suggested in evaluating restoration programmes (Lambeck 1997; Andelman & Fagan 2000; Heywood & Iriondo 2003), although the usefulness of these surrogates as indicators of regional biodiversity has been debated (Andelman & Fagan 2000; Lindenmayer *et al.* 2002).

More recently, ecological restoration has shifted towards restoring whole communities (Ehrenfeld 2000; Menninger & Palmer 2006). Yet determining fundamental boundaries that differentiate communities from ecosystems have further complicated restoration goals (Simberloff 1990; Morin 1999), whilst the organisation of communities and ecosystems themselves remain unclear (Paine 1988; Peters 1988). For example, communities have been considered as collections of independent populations exhibiting collective properties (i.e. properties equivalent to the sum of the individual components), though the more accepted view suggests communities to be intricately and obligatorily linked populations such that they exhibit emergent properties rather collective ones (Simberloff 1990; Brown 1995; Levin 1998). This latter definition of interacting communities however has particular implications for restoration, as their emergent properties, by definition, cannot be predicted solely by the sum of their components, their effects typically greater than collective properties (Cardinale & Palmer 2002; Cardinale *et al.* 2002). Thus the restoration of the community components alone cannot guarantee the restoration of a fully interacting community itself.

Not knowing what level to restore to is further complicated by the fact that this 'fuzzy target' is also a 'moving target'(Simberloff 1990). That is to say that the ecological community that is to be restored is one that is constantly changing as a

consequence of a number of abiotic and biotic factors, naturally or anthropogenically induced, working at both temporal and spatial scales (Southwood 1977; White & Walker 1997; Menninger & Palmer 2006). Consider the pollinators of the community for example. Their populations will fluctuate over the course of the flowering period (Gross & Werner 1983; Herrera 1988), whilst their distribution will be dictated to some extent according to the phenology of flowering in the community (Gross & Werner 1983; Ashman & Stanton 1991). Pollinator communities will vary within (Herrera 1988; Cane & Payne 1993), and between seasons (Pettersson 1991; Fishbein & Venable 1996), or change completely in composition over the lifetime of the longer-lived plant species (Petanidou & Vokou 1993; Roubik 2001).

In terms of spatial variation, pollinator communities will vary locally according to plant population size and patch distribution (Thomson 1981; Westphal *et al.* 2003), and regionally according to the fragmentation of the matrix (Rathcke & Jules 1993). Furthermore, complications may arise at the landscape context, with different pollinators perceiving the landscape (Steffan-Dewenter 2002; Tscharntke & Brandl 2004) and their surrounding matrix (Jules & Shahani 2003) at different spatial scales. These pollinators will in turn be potential hosts to a wide range of parasites and parasitoids. With hosts varying their phenology spatially and temporally, their parasites and parasitoids in turn will require flexibility in their own behaviour and physiology in locating their hosts, timing their oviposition, adjusting the length of host-bound development and timing their emergence to coincide with locating potential mates (Godfray 1994; Schmid-Hempel 1998). Thus variation in both time and space are crucial factors affecting the strength and diversity of interactions within all communities.

Reference information is critical in defining realistic restoration goals. Firstly, reference sites provide baseline data against which the restoration programme can be compared when a) assessing its restoration potential, b) determining what to restore, and c) evaluating the trajectory of the restoration over time (White & Walker 1997). Secondly, it is crucial to understand the inherent natural variation over both spatial and temporal scales of the reference target community, before determining the degree of variation that is acceptable in the restored community (Pickett 1976; Aronson & LeFloch 1996; Palmer *et al.* 1997; White & Walker 1997). Indeed the resilience of a system can be seen as a function of its temporal and spatial variation (Tilman *et al.* 1996), with many degraded systems lacking the natural variation

needed to buffer their communities from future perturbations, leaving them unsustainable (Pedroli & Harms 2001; Baron *et al.* 2002).

Replicating reference and restoration sites in both time and space are therefore important in determining the goals in restoration and in evaluating its success. More often than not however restoration projects lack adequate replication (Simberloff 1990; Osenburg *et al.* 2006). This may be due to the application of non-uniform treatments, or more commonly because suitable reference sites for highly threatened systems no longer exist (Cairns & Heckman 1996; Michener 1997). Yet although restoration sites have the potential to be used as large-scale experiments for the development and testing of basic ecological theory e.g. concepts such as community structure and function (Bradshaw 1987; Palmer *et al.* 2006), only more recently are researchers undertaking more rigorous scientific approaches in assessing ecological restoration (Osenburg *et al.* 2006).

Evaluating restoration success

> *"For many who have tried to restore viable self-sustaining populations to the wild, there has come a great sense of humility and wonder at the complexity of systems that have a deceiving appearance of simplicity"*
>
> (Maschinski 2006)

Ecological restoration, as defined by the U.S. National Research Council earlier in this chapter, requires both ecosystem structure and function to be returned such that ecosystem processes can operate effectively. Yet many restoration programmes in the past have focused more on reinstating the superficial structure rather than the function of that system (Simberloff 1990; Handel 1997; Palmer *et al.* 1997; Forup *et al. in press*). For example, past authors have used vegetative structure (Walters 2000; Wilkins *et al.* 2003), seed bank characteristics (Mitchell *et al.* 1999; Pywell *et al.* 2002; Walker *et al.* 2004), or species diversity (Reay & Norton 1999; Passell 2000: McCoy & Mushinsky 2002) as their indicators of restoration success. Aside from confirming that their respective restoration projects are progressing in an upward direction along the restoration trajectory, little can be inferred about the reinstatement of function to that community from this type of data. Recall that community properties are not simply the sum of their individual components. Thus

although restoration may have successfully resulted in the return of species diversity to the restored community, it is no guarantee that the processes those species are involved in have been re-established (Zedler 1993; Zedler & Callaway 1999).

Consequently many restoration projects result in only partial restoration or 'rehabilitation' of communities and systems, relying instead upon the resilience of the system itself to reinstate function on its own accord. By reinstating this basic community structure, e.g. the vegetative structure of the target habitat type, it was assumed that the organisms associated with such habitats would be expected to arrive and establish themselves over time (Anderson 1995; Handel 1997; Palmer *et al.* 1997; Ormerod 2003). Referred to as the 'Field of Dreams hypothesis' (Palmer *et al.* 1997), this remains poorly tested, but relies on a variety of factors including the ability of species to disperse, recruit, and reinstate their interactions with other species, thereby contributing to the overall 'functional redundancy' of that community. This 'functional redundancy' refers to the ability of different species to play functionally similar roles (Walker 1992; Lawton & Brown 1993), an essential attribute of functioning and self-regulating ecosystems (Naeem 1998). Thus the functional role of a species that has yet to re-establish in the restored community, can be compensated for by those species that have been reinstated, thereby allowing the for the passive return of essential ecological processes over time, despite an initially less complex community (e.g. pollination (Corbet 2000; Kearns 2001); soil processes (Rhoades *et al.* 1998)).

If functional redundancy is known to be high, then it is possible to set a minimal level for the restoration of species diversity that ensures the gradual establishment of ecosystem functioning (Palmer *et al.* 1997). Such 'thresholds' have been tested theoretically (Lundberg & Ingvarsson 1998; Fonseca & Ganade 2001), although in reality data to support a suggested threshold level remains scarce (Lambeck 1997; Palmer *et al.* 1997), whilst potential surrogate species may actually behave differently (Montalvo *et al.* 1997). More generally, the factors involved in determining the extent of a community's functional redundancy remain poorly studied with regard to their role in restoration (Pywell *et al.* 2003; Walker *et al.* 2004; Memmott *et al.* 2006). Restored habitats that fail to reinstate function together with the basic structure of the community have therefore been referred to as the 'living dead' (Handel 1997), whereby the inadequate restoration of the ecological processes of that community results in that community failing to be self-sustaining.

For example, restoring a community's basic vegetative habitat without their required pollinators would limit it to a single generation, or result in inbreeding depression as a consequence of self-fertilisation or vegetative propagation (Dejong *et al.* 1993; Barrett 2003).

More recently, the evaluation of restoration success has taken a more integrative approach by including a range of attributes as measures of restoration success. In particular, the Society for Ecological Restoration International (SER) in their 2004 primer (SER 2004), highlighted nine attributes of fully restored ecosystems. These included parameters of community structure that are easily comparable to their reference communities: species composition, diversity, distributional abundance, the presence of essential functional groups of species, the low abundance of ruderal and non-native species, and the ability of the physical environment to sustain the nesting and reproduction requirements of the species present. Parameters indicating the return of function to restored sites included: the absence of signs of dysfunction, the integration and interaction of the restored ecosystem with the landscape, a resilience to natural disturbances, and evidence of self-sustainability and persistence through natural variation or environmental change. Yet these latter attributes of community function are considerably harder and more costly to measure, and have thus been frequently ignored in the evaluation of the success of restoration projects (Ruiz-Jaen & Aide 2005). More recently however, research is beginning to focus on the restoration of function (Falk *et al.* 2006), evaluating ecological processes and interactions such as soil processes (e.g. Rhoades *et al.* 1998), parasitism (e.g. Huspeni & Lafferty 2004), and pollination (e.g. Forup & Memmott 2005b; Forup *et al. in press*).

Restoring ecological interactions and networks

> *"It is interesting to contemplate an entangled bank, clothed with many plants of many kinds, with birds singing on the bushes, with various insects crawling through the damp earth, and to reflect that these elaborately constructed forms, so different from each other, and dependent on each other in so complex a manner, have all been produced by laws acting around us."*
>
> (Darwin 1859)

In his concluding paragraph of The Origin of Species (1859), Darwin intuitively noted that species do not and cannot exist in isolation, but rather within an "entangled bank" of interactions. Indeed species interact with each other in a variety of ways, including competitive, trophic, and mutualistic interactions. Such interactions structure ecological communities (Hairston *et al.* 1960; Paine 1969; Stachowicz 2001; Chase *et al.* 2002; Cardinale *et al.* 2002; Bruno *et al.* 2003), and essentially drive important ecological processes in the ecosystem (Memmott *et al. in press*). For example, insects that act as decomposers together with the soil biota promote nutrient turnover and decomposition; pollinators transfer pollen between plants upon foraging for floral resources; frugivores transfer and disperse seeds; predators, parasitoids and parasites provide biological control. In doing so, species interactions thereby provide essential ecosystem processes and services that are necessary for the self-regulation and persistence of the community (Handel 1997; Palmer *et al.* 1997; Vander Zanden *et al.* 2006).

Yet the capacity for ecosystems to provide such services is rapidly being eroded, if not surpassed, as a consequence of continual anthropogenic pressures altering their structure and function (Vitousek *et al.* 1997a; Palmer *et al.* 2004). Restoring ecological interactions to degraded systems can facilitate the return of functional processes, and indeed restoration ecologists now recognise species interactions as key attributes of successfully restored systems (Menninger & Palmer 2006). Below I outline the important economic value of ecological interactions, introduce those species interactions that will be considered in this thesis, and briefly introduce the usefulness of a network approach as a tool in evaluating restoration success, with regard to the reinstatement of ecological interactions to restored systems.

The value of ecological interactions

Ecological interactions have been considered the currency of ecosystem services (Memmott *et al. in press*). Such services are essentially 'gratis', representing 'natural capital' that are of direct benefit to mankind (Hawken & Lovins 1999), and include services such as erosion control, nutrient recycling, pollination, seed dispersal, and pest control (Daily 1997; Norberg 1999).

Recently, the true economic value of ecosystem services has been realised, following costly attempts to replace them or mitigate the impacts that arise following their loss (reviewed in Pimentel *et al.* 1997; Balmford *et al.* 2002). Indeed Costanza *et al.* (1997) valued the global natural capital at a conservative average of US$33 trillion – a value almost twice the global Gross National Product (GNP). More recently in 2006, four ecosystem services provided by insects alone (pollination, pest control, dung burial and recreation) were estimated at a total value per annum of over USD$57 billion (Losey & Vaughan 2006). Restoring ecosystems to reinstate their capacity for the provision of services is therefore valuable and economically viable (e.g. van Wilgen *et al.* 1996; Higgins *et al.* 1997; PCAST 1998; Heal 2000).

Plant-pollinator interactions

"silent springs and fruitless falls go hand in hand"

(Carson 2002)

Pollination is an essential ecosystem service responsible for the production of up to 30% of food incorporated into our diet (McGregor 1976 cited in Losey & Vaughan 2006), and therefore of high monetary value, globally estimated at US$12 billion (Costanza *et al.* 1997). Furthermore, up to 90% of all flowering plants are dependant upon animals for cross-pollination (Buchman & Nabhan 1996), 67% of which are pollinated by insects alone (Kearns & Inouye 1997). Yet it has been estimated that over the next 300 years, up to half a million insect species may become extinct (Miller 1993; Mawdsley & Stork 1995), whilst in 1999, the São Paulo Declaration on Pollinators highlighted the serious declines in the number of native pollinator species throughout America and Europe (Dias *et al.* 1999). This disruption to pollinator systems, globally widespread, has thus been coined "the pollinator crisis" (Buchman & Nabhan 1996).

Such a "crisis" has been initiated and worsened by continual anthropogenic pressures impacting upon the pollinators themselves (Rathcke & Jules 1993; Kearns *et al.* 1998; Spira 2001; Tscharntke & Brandl 2004). Habitat fragmentation can impact upon pollinator diversity (Aizen & Feinsinger 1994), pollinator abundance (Donaldson *et al.* 2002; Lennartsson 2002), and foraging distances (Powell & Powell

1987; Gathmann & Tscharntke 2002), whilst patch isolation and edge effects can impact upon seed set (Jennersten 1988; Steffan-Dewenter & Tscharntke 1999), and pollinator behaviour (Montgomery *et al.* 2003). Other pressures such as fire (Potts *et al.* 2001), pesticide use (Johansen 1977; Richards 1993), grazing (Kearns *et al.* 1998), and the introduction of non-native species (Kearns & Inouye 1997; Steffan-Dewenter & Tscharntke 2000), have all contributed to the pollinator crisis.

Despite the global impacts of declining pollinators on pollination services (Kearns & Inouye 1997; Allen-Wardell *et al.* 1998), plant-pollinator interactions have still received relatively little attention in restoration ecology (Neal 1998; Memmott 1999). Yet the restoration of degraded terrestrial systems more often than not involves restoring degraded and isolated plant populations. Pollinators can maintain self-sustaining populations through facilitating seed set, allowing gene flow between plants necessary in maintaining its genetic diversity (Kearns *et al.* 1998; Spira 2001). The reinstatement of plant-pollinator interactions to degraded systems is therefore crucial in ensuring the survival of the floral community beyond a single generation, and essential if ecological restoration is to succeed in re-establishing a self-sustaining community of plants and pollinators (Forup *et al. in press*).

Host-parasite interactions

> *"So, Nat'ralists observe, a Flea Hath smaller Fleas that on him prey, And these have smaller Fleas to bit 'em, And so proceed ad infinitum"*
>
> (Swift 1733)

Research increasingly sees parasites as important regulators in the ecology and evolution of their individual hosts, host populations, and even entire communities (Schmid-Hempel 1998). For example, hosts are constantly adapting to their parasites and vice versa through behavioural, genotypic and phenotypic adaptive responses (Horwitz & Wilcox 2005). Also, the capacity of the host to harbour natural enemies (Flatt & Scheuring 2004), and the role for both parties of genetic variation in mediating parasite transmission (Morand *et al.* 1996; Kraus & Page 1998; Schmid-Hempel 2000), and in determining parasite virulence (Lenski & May 1994; Nowak & May 1994; Ebert & Hamilton 1996), further stabilise and regulate host populations. Parasites therefore play an important role in structuring host communities (Dobson &

Hudson 1986; Price *et al.* 1986; Minchella & Scott 1991), whilst also mediating a range of competitive and predatory interactions (Price *et al.* 1986; Prenter *et al.* 2004). Furthermore, parasites have been shown to influence the overall biodiversity and functioning of communities (Mouritsen & Poulin 2002; Montoya *et al.* 2003). Such a diversity of roles is not surprising given the ubiquity of parasites in nature, with almost 100% of species parasitized (Esch & Fernandez 1993), and the richness of parasitic species exceeding that of free-living species (Price 1980). Furthermore, the very nature of parasites excludes them from existing in isolation due to their dependence on their host for survival (Smyth 1994). Indeed in the previous quote by Swift (1733), the recognition that hosts are parasitized by parasites that are themselves potential hosts to further parasites and so on, illustrates an early recognition of the potential complexity of host-parasite interactions within the broader network of ecosystems.

Parasite-host interactions in communities are shaped by both the environment of the host itself and the host's natural environment. Parasites are therefore generally more susceptible to extinction than the hosts themselves, being subject to perturbations that impact on both environments rather than one alone (Didham *et al.* 1998; Holt *et al.* 1999). Consequently, not only can parasites provide information on their hosts (such as host diversity, abundance, and trophic position, and therefore food web structure), but their persistence over time can act as an indication of repeated, ecological and seasonal patterns of both their host and their environment (Marcogliese & Cone 1997; Marcogliese 2003; Marcogliese 2005). Thus the value of using parasites as effective bioindicators of environmental degradation is increasingly being realised (Cone *et al.* 1993; Marcogliese 2003; Valtonen *et al.* 2003; Marcogliese 2005).

Yet despite this parasites are frequently excluded from food web studies (Huxham & Raffaelli 1995; Marcogliese & Cone 1997; Marcogliese 2003; Dobson *et al.* 2006). Their obscurity, effectively being "hidden players" of communities (i.e. the cryptic invertebrates, microbes and disease (Thompson *et al.* 2001)), may have limited past research, such that their omission in community ecology today is a consequence of historical biases. However it is now known that their exclusion from food webs can alter fundamental properties of network structure and function (Schoener 1989; Freeland & Boulton 1992; Huxham & Raffaelli 1995; Huxham *et al.* 1996; Lafferty *et al.* 2006). Incorporating such hidden players into community

studies is now a key direction in research, and indeed restoration ecologists are also beginning to acknowledge the importance of their restoration (Menninger & Palmer 2006).

Alien (non-native) species interactions

The last 200 years has seen an exponential rise in the frequency of both intentional and accidental introductions of non-native species, primarily due to the increase in human mobility and commerce (Lovei 1997; Mack *et al.* 2000; Levine & D'Antonio 2003; Pauchard & Shea 2006). The arrival and subsequent establishment of alien species into new habitats, usually far beyond the native range of their distribution (Hodkinson & Thompson 1997; Mack *et al.* 2000; Richardson *et al.* 2000), is a global phenomenon: Vitousek *et al.* (1997b) found that up to 35.8% and 82.8% of all naturalised vascular plant species on continental areas and islands respectively were established alien species. This ability of alien species to overcome geographical, abiotic and biotic, reproductive, and dispersal barriers of their new environment qualifies them as successful invaders (Vermeij 1996; Richardson *et al.* 2000), and their proliferation and persistence outside their natural range has huge implications for native biodiversity (Drake 1989; Vitousek *et al.* 1997a; Chapin *et al.* 2000).

Alien invasive species negatively impact upon communities and ecosystem processes, primarily by altering and competing for resources otherwise available to native species (Vitousek *et al.* 1987; Von Holle *et al.* 2006), by affecting disturbance regimes and the physical structure of the ecosystem (Bertness 1984; D'Antonio & Vitousek 1992), and by acting as predators and parasites of native species (Anagnostakis 1987; Taraschewski 2006). Furthermore, alien invasive species can contribute to the loss of ecosystem services by interfering with native mutualisms (Traveset & Richardson 2006) and altering native interaction webs (D'Antonio & Vitousek 1992; Memmott & Waser 2002; Morales & Aizen 2002; O'Dowd *et al.* 2003; Traveset & Richardson 2006). Ultimately therefore, alien invasive species accelerate native species extinctions (Wilcove *et al.* 1998; Sax & Gaines 2003), homogenise the global biota (Lovei 1997; Sax & Gaines 2003), and thereby threaten global biodiversity (Wilson 1992; Wilcove *et al.* 1998).

Restoration ecologists are therefore faced with restoring degraded communities against a backdrop of an exotic-dominated world (D'Antonio & Chambers 2006), such that the restoration of habitats and ecosystems increasingly involve managing for alien invasive species. Firstly, restoration programmes typically involve the removal of alien invasive species. This may involve the removal of species that contributed to the degradation of the system in the first instance, or indeed the removal of species that colonise and invade the habitat during the restoration process itself (Anderson 1995; D'Antonio & Chambers 2006). Secondly, restored habitats are themselves highly susceptible to invasions, as their communities lack the ability to exclude alien invaders. This may be due to the availability of unoccupied niches during the early stages of restoration, where, for example, the presence of open space and unused nutrients during the pioneer stages of the restored native vegetation may facilitate the colonisation of alien plant species (Anderson 1995). Alternatively, the simplification of species interactions in degraded or partially restored communities may reduce their resistance to invasions. Indeed the complex array of interactions between native species can mediate species extinctions (Memmott *et al.* 2006) and the impacts of alien invasions (Prenter *et al.* 2004), whilst providing inherent resilience to the community itself (Sole & Montoya 2001; Dunne *et al.* 2002; Ives & Cardinale 2004; Memmott *et al.* 2004).

To manage for invasive exotics in restored systems, restoration ecologists are therefore faced with implementing effective removal programmes (i.e. a top-down control approach, whereby the invasive species is reduced or removed), as well as preventative measures (i.e. a bottom-up control approach, whereby the properties that contribute to resistence against invasions are restored) (McEvoy & Coombs 1999; D'Antonio & Chambers 2006). Understanding the degree to which an alien invasive species can impact upon the establishment of species already present, or upon future successional or colonisation events of both alien and native species at restored sites, is critical if ecologists are to fully understand the mechanisms that drive species invasions. Only then can the processes that need to be overcome for restoration to be successful, be identified effectively (Zavaleta *et al.* 2001; Levine *et al.* 2003; D'Antonio & Chambers 2006).

Interaction networks

Early studies of interaction networks were in the form of food webs, documenting predator-prey or host-parasite relationships and thereby linking species from a number of different trophic levels into a community (e.g. Hairston *et al.* 1960; Paine 1966; Menge & Sutherland 1976). Food web theory provides an important analytical tool for the study of the structure or topology of food webs, and the important characteristics they might infer. For example, the strength, number and distribution of species interactions of a food web can be analysed (Paine 1980; Jordano 1987; Pimm *et al.* 1991; Paine 1992; McCann *et al.* 1998), providing measures such as connectance (the proportion of the total number of possible interactions between species that actually occur (Martinez 1991)) and linkage density (the number of links per species (Pimm *et al.* 1991)). Such measures provide an indication of the complexity of the community or ecosystem studied (Pimm & Lawton 1980; Yodzis 1980; Jordano 1987; Martinez 1992; Raffaelli & Hall 1992; Brualdi & Sanderson 1999; Bascompte *et al.* 2003; Kondoh 2003; Krause *et al.* 2003).

More recently, the use of food web theory in analysing the characteristics of a more diverse suite of interaction networks is being realised. Aside from analysing negative interaction networks such as predation and competition, food web theory is now being incorporated into interaction networks that instead involve mutualistic associations such as plant-pollinator networks (Jordano 1987; Fonseca & Ganade 1996; Memmott 1999; Dicks *et al.* 2002; Forup & Memmott 2005b; Bascompte & Jordano 2006). Furthermore, the study of interaction networks rather than specific interactions in isolation, has furthered our understanding of network properties such as those that provide stability to networks (Sole & Montoya 2001; Dicks *et al.* 2002; Dunne *et al.* 2002; Melian & Bascompte 2004). Such properties have been found to govern a network's resilience to both primary and secondary species extinctions (Dunne *et al.* 2002; Ives & Cardinale 2004; Memmott *et al.* 2004; Sole & Montoya 2006).

Consequently the use of interaction networks as an important analytical and predictive tool in the conservation of ecological interactions is increasingly recognised (Memmott *et al.* 2006; Memmott *et al. in press*), particularly as they are continually threatened by the loss of component species and the addition on non-native species (Kearns et al 1998). Interaction networks have thus been used to study

and predict the impacts of pollinator extinctions (Memmott *et al.* 2004), apparent competition (Morris *et al.* 2004), and habitat modification (Tylianakis *et al.* 2007). Yet with regard to the field of restoration ecology, the contribution of interaction networks and food web theory to the planning and evaluation of ecological restoration is only just becoming apparent (Vander Zanden *et al.* 2006; Forup *et al. In Press*). In particular, the application of an interaction-driven approach has the potential to identify key subsets of ecological interactions that are important in determining the outcomes of restoration (Zavaleta *et al.* 2001; Roemer *et al.* 2002), whilst also providing a useful tool for evaluating the restoration of community function.

Aims of this study

The work presented in this thesis primarily aims to determine the potential for the restoration of key ecological interactions following ecological restoration programmes. In doing so, I focus particularly on the plant and pollinator interactions and the host and parasite interactions of communities undergoing restorative management to evaluate restoration success. A food web approach was used to analyse both the structural and functional characteristics of these interaction networks for the comparison of restored communities against baseline, reference communities. Finally I investigated the potential for positive interactions between non-native, invasive species, with regard to understanding how to manage such interactions effectively within restoration programmes.

1.2 Thesis structure

Chapter two focuses on the plant and insect communities of restored heathlands located in south Dorset, UK, following eleven and fourteen years of restorative management. Here I evaluate the return of both the structural and functional parameters of the pollination network, using the reinstatement of the plant-pollinator interactions as a surrogate measure of returning community function. A network approach was used to compare network topology between four pairs of restored and ancient heaths over two years.

In Chapter three, I use the same system as in the previous chapter, expanding my approach to include an assessment of the host – parasite interactions in the community. Here the bumblebee flower visitors and their natural enemy community were the focus of this study, using interaction networks to compare six pairs of restored and ancient heathland communities. Here I determine whether all levels of the tri-trophic community were reinstated to restored heathlands.

In Chapter four, I examine the prevalence and patterns of parasitism in bumblebees of the heathland communities studied in Chapter three, to both update the relatively depauperate knowledge of bumblebee parasitism in the UK, and to provide baseline data on the extent of parasitism for bumblebee host species on British, dry lowland heathland.

In Chapter five, I assess the interactions that occur between two alien invasive plant species *Linaria vulgaris* and *Cirsium arvense*, in the alpine wildflower meadows of the Rocky Mountains of Colorado, USA. I ask what potential there is for facilitative interactions to occur between these two alien invasive species to determine which processes need to be overcome in order to manage their invasive spread.

Finally in Chapter six, I bring together the main findings of the thesis. I consider their application in the wider context, discuss the importance of incorporating the study of ecological interactions into restoration ecology, and outline potential directions for future research.

CHAPTER TWO

The restoration of plant-visitor interactions in dry lowland heathland

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CHAPTER TWO

The restoration of plant-visitor interactions in dry lowland heathland

Summary: *Ecological restoration has the potential to replace essential ecosystem services, yet many restoration projects tend to focus solely on reinstating structure, expecting function to return to the community on its own accord. However this is neither guaranteed nor adequately assessed, such that many restoration projects fail to reinstate functioning or self-sustaining communities.*

This chapter assesses the return of both structural (species abundance, diversity and composition) and functional parameters (the reinstatement of species interactions) of plant-pollinator communities to restored heathlands, following eleven and fourteen years of restoration. A network approach was used to evaluate their restoration success by mapping species interactions into interaction networks, allowing the four pairs of restored and ancient reference communities to be compared in both sampling years.

Both the flower and insect communities were found to be successfully reinstated at restored heathland following fourteen years of restorative management. However restored heaths were found to be functionally less complex than their paired reference sites with regard to the density and proportion of interactions realized between their species, whilst also experiencing a lag in floral resource availability. With regard to species composition, adjacent heathlands did not share any more species than with more distant heaths, suggesting that the requirement for restoration sites to be adjacent to ancient communities is unnecessary regarding the re-establishment of insect visitors. Furthermore, a large proportion of species were found to vary markedly over both time and space, with most of this attributed to temporal variation. However a great majority of visitors to heaths, represented by only a small proportion of species, were consistent over both space and time, suggesting the successful reinstatement of core pollinator guilds to restored heathlands.

2.1 Introduction

Heathlands are man-made habitats that arose during the Bronze Age 3000 - 4000 years ago, and are representative of former traditional agricultural systems that dominated Western Europe up until the 18th Century. However much heathland was converted to forestry or lost to urban expansion following the intensification of agriculture (Moore 1962; Walker *et al.* 2004). Consequently the UK has seen the loss of over 80% of the original extent of ancient heathland, with 40% of these losses having occurred since the 1950's (Symes & Day 2003). Today remnant fragments are further threatened by the continual encroachment of scrub and succession to pine at a rate of 1.7% per year (Rose *et al.* 2000), otherwise kept in check through intensive conservation management that have since replaced past traditional agricultural practices.

Heathlands represent one of the few remaining semi-natural habitats in Europe, providing essential refugia for many rare and protected reptile and bird species (Bibby 1978; Webb & Haskins 1980; Spellerberg 1989; Liley & Clarke 2003), as well as supporting many endangered invertebrate species (Kirby 1992). Consequently heathlands are high-priority habitats for biodiversity and wildlife conservation, and are protected under the EU Habitats Directive. Moreover, due to the National Lowland Heathland Biodiversity Action Plan, the UK Government has committed itself to the restoration of 6000 hectares of heathland by 2010, alongside the conservation management of the current 58,000 hectares that exist (Biodiversity Action Plan website). Much of this restoration began in the mid 1990's, predominantly on habitats close to existing heathland that were themselves heathland previously, but had since been converted to other land uses (e.g. agriculture or forestry).

The restoration of heathland in the UK has been considered to be most successful on former conifer plantations (Pywell *et al.* 2002; Walker *et al.* 2004). However the success of heathland restoration has frequently been defined by the speed at which the heathland floral community re-establishes, with the persistence of the seed bank and heathland soils present in the restored area used as a predictor for the longer-term success of the restored heathland (Mitchell *et al.* 1999; Pywell *et al.* 2002; Walker *et al.* 2004). This focus on restoring habitat structure, whist paying

minimal attention to the restoration of ecological function, is common in ecological restoration (Simberloff 1990; Handel 1997; Forup *et al. in press*). Yet community function is essential for the self-maintenance and self-regulation of communities, and is driven by the ecological processes and services arising from interactions that occur between species of that community (Loreau *et al.* 2001; Naeem 2002). Without such interactions, a community under restoration is unlikely to persist beyond the restoration programme (Falk *et al.* 2006). Instead, restoration programmes rely on passive rehabilitation to return function to the restored community over time (Anderson 1995; Ormerod 2003). However this is a slow process, dependent upon the re-establishment and re-assembly of interactions between species - processes which remain poorly understood in the context of restored systems (Simberloff 1990; Handel 1997; Pywell *et al.* 2002; Walker *et al.* 2004; Memmott *et al.* 2006). Thus the return of function to a restored habitat is far from guaranteed or adequately assessed in restoration (Zedler 1993; Neal 1998; Zedler & Callaway 1999).

Furthermore, environmental stochasticity and variation over both time and space will tend to act more directly upon the structure of a community (i.e. species diversity, abundance and composition), than upon its function (e.g. processes and services such as pollination and pest control). Species abundance and composition for example are directly affected by environmental conditions and other biotic factors such as regional and local species pools and disturbance regimes (White & Walker 1997; Potts *et al.* 2003; Price *et al.* 2005). However the impact of such factors on the ecological processes and services can be effectively buffered by a community due to its inherent functional redundancy. Such redundancy occurs in intact communities, as they are able to compensate for any missing or degraded species by replacing them with other, functionally similar species from their species pool (Walker 1992; Naeem 1998). Consequently the evaluation of restoration success through measuring community structure alone can be misleading, particularly if the assessment of the restoration is limited temporally (i.e. assessing restoration at only one point in time), or spatially (i.e. assessing restored sites against too few or inappropriate reference sites). Both ecological structure and function should therefore be assessed simultaneously to determine the potential for community persistence and resilience to natural perturbations (Falk *et al.* 2006; Forup *et al. in press;* Memmott *et al. in press*).

As the interactions between species are essentially the currency of ecosystem services, assessing their reinstatement to restored communities can provide an indication of the re-establishment of ecosystem processes and services. Indeed it is the reinstatement of these services that remains one of the most challenging areas in restoration ecology. Yet the use of interactions between species as potential indicators of restoration success or habitat quality is rarely adopted (Tscharntke *et al.* 1998; Forup *et al. In Press*). This study evaluates the reinstatement of interactions between plants and their potential insect pollinators to communities undergoing ecological restoration. By taking a food web approach, these interactions can be mapped into interaction networks and analysed for entire communities (Jordano 1987; Memmott 1999), thereby allowing a more holistic approach to the assessment of community restoration. For example, by comparing interaction networks between restored and reference communities, not only can aspects of community structure (such as species composition and abundance) be analysed, but the interactions themselves can inform restoration ecologists of the complexity of their restored communities relative to their reference sites. Such measures of complexity are particularly important as they can determine a community's resilience to environmental perturbations such as habitat loss and species extinctions (Sole & Montoya 2001; Dunne *et al.* 2002; Ives & Cardinale 2004).

This study uses quantitative interaction networks to evaluate the restoration of the plant and pollinator community to heathlands in south Dorset, England. In this chapter I focus on the following four questions:

1) Are the interaction networks of ancient heaths more complex than restored heaths?

2) Have the flower and insect visitor communities been reinstated in restored heathlands?

3) Do the pollinators of restored heaths originate from their adjacent, ancient heaths?

4) Did the pollinators of restored heaths become more similar in composition to ancient heaths over time?

2.2 Methods

Using a paired design with both spatial and temporal replication, this study evaluates the restoration of pollinator communities to restored heathlands. The reinstatement of pollinator diversity, abundance and pollinator interactions to restored systems is crucial to ensure the return of pollination services, these being essential for the reproduction of the vast majority of flowering plants and the maintenance of genetic diversity in the seed bank (Neal 1998). Here, four pairs of ancient and restored heathland communities were compared for their plant and visiting insect communities in 2001 and 2004 following eleven and fourteen years of restorative management respectively. The abundance and diversity of insect visitors were taken as a measure of returning community structure, whilst the flower–visitor interactions were quantified to provide an indication of the return of ecological function.

Study habitat

Typical dry, lowland heath exist on impoverished soils that support a species-poor plant community, primarily dominated by ericaceous shrubs and dwarf gorses interspersed with lichens, bryophytes, and scrub, usually under sparse tree cover. In Dorset, 96% of the heathlands have been designated as Sites of Special Scientific Interest (SSSI's) under the Wildlife and Countryside Act 1981. Currently 20% of the total international area of dry lowland heathland is located in Britain (Auld *et al.* 1992), of which 12% (6000 ha) is found in Dorset. By concentrating the sites within this region the influences of changing geology, soil type and climate on the composition of heathland communities were minimised.

Site selection

Four ancient (A1-A4) and four restored (R1-R4) heathland fragments located in the Purbeck District in south Dorset, England, were paired according to geographic location, management intensity and management technique (e.g. grazing and burning) by M.L. Forup in 2001 (Table 2.1; Figure 2.1) (Forup 2003). Plots within a

pair were separated by distances ranging between 0.5 to 3.6 kilometres, with pairs separated by 2 to 12 kilometres. The variation in separation distances were as a consequence of the continual fragmentation of remnant heathland by urban expansion. Ancient heaths of these pairs acted as controls against which restored sites were compared. The ancient heathland sites chosen were remnant fragments of the original heathland extent that reached its maximum as a single, vast landscape over 300 years ago (Symes & Day 2003). Today these remaining fragments vary significantly in quality, size and locality, as a consequence of urban expansion and conversion to agriculture (Walker *et al.* 2004). Ancient sites were selected to be similar in fire history and management regime (i.e. grazing, rotational heath and gorse maintenance, and scrub control such as pine felling, rhododendron spraying, and bracken control), and all were more than 250 years old.

Restored heathland sites were heaths that were once originally part of the single, continual heathland landscape, but underwent afforestation with pine (predominantly with Scots pine, *Pinus sylvestris*, and Maritime Pine, *Pinus pinaster*) in the 1950's. Typically, managers choose to restore formally afforested heathland rather than former arable land (Walker *et al.* 2004), as their more suitable soils and existing relict seed banks promote the regeneration of the heath flora and the expansion and linkage of the ancient extant fragments (Symes & Day 2003). Their restoration back to heath began in the early 1990's in the UK, with an aim to increase linkage to existing remnant fragments and the overall heathland area (Anon 1995). Such programmes primarily involve tree felling, the removal / loosening of humus and topsoil, and scrub control (Auld *et al.* 1992).

Sampling protocol for network construction

Data collection was conducted during the summer of 2001 by M.L. Forup and in 2004 by K.S.E. Henson on the four pairs of restored and ancient heathland sites. Sampling was conducted every 17 – 21 days between late April and the end of September in 2001 by M.L. Forup, and between early May and the end of September in 2004 by KS.E. Henson. This resulted in eight sampling sessions per site per year. Slightly different sampling methods were used in the two years, as the methods in 2004 were changed to conform to methods used in other ongoing pollination

projects. In 2001, circular sampling plots each of 100m radius were established by M.L. Forup. At the start of each sampling session, two 100m transects radiating out from the centre of the plot were randomly selected. In 2004, these plots were re-established as 100m x 100m square plots, their central point being the same as the central point of the circular plots previously established in 2001. For each of the square plots, one of the four 100m edges was established as the baseline. Ten 100m long transects spaced 10m apart along this baseline were divided into two blocks of five transects each. At the beginning of each sampling session, one transect from each block was randomly selected for sampling. Although plot sizes were larger in 2001 (31,416m^2 in 2001 vs. 10,000m^2 in 2004), the actual area sampled for flora and fauna was the same for both years (2 x 100m transects x 8 sampling sessions).

1) Floral sampling:

In 2001 and 2004, two 100m long transects were surveyed at each plot per sampling session for flowers. Six quadrat recordings were taken at 15m intervals along each transect, and the total number of flowers and number of floral species within the quadrat area were recorded. For the dominant plant species *Erica tetralix, Erica cinerea,* and *Calluna vulgaris*, floral abundance during peak bloom was estimated by multiplying the mean number of flowers per floral spike by the number of floral spikes present. Mean floral abundance and species richness were calculated per m^2.

2) Insect sampling:

The two 100m transects sampled for flora at each plot per sampling session were also used to sample visiting insects in both 2001 and 2004. Sampling only took place under calm, warm and dry conditions, and were rotated through morning, early afternoon or late afternoon sessions over the season for both sampling years. This was done to avoid differences that may have emerged due to variation in insect activity over the course of a day. Each transect was walked twice and all insects found interacting with a flower 1m either side of the transect line were captured using a sweep net, or directly into a glass vial containing ethyl acetate (lethal relaxant). Queen bumblebees were not collected to minimise impact upon colony densities. The floral species foraged on by the insect sampled was recorded. The area sampled for insects was consistent for both sampling years at 2 x 200m^2 per transect.

All insects were identified to species or morphotype by taxonomists at the National Museum of Wales, with exception to bumblebees in 2004 where identification was done by K.S.E. Henson using Prys-Jones & Corbet (1991) and Benton (2000). Workers of *Bombus lucorum* and *B. terrestris* were grouped as *B. luc/terr* in both years due to the difficulties in separating these species, similar to Dicks *et al.* (2002).

Data analyses

Q1) Are the interaction networks of ancient heaths more complex than those of restored heathlands?

The visitation data from both 2001 and 2004 were used in the construction of quantitative flower visitation networks for each of the heathlands sampled. These networks were drawn using a program written in Mathematica™. Food web statistics calculated for the plant-visitor networks include: 1) the maximum number of links possible (calculated as i*p where 'i' and 'p' are the number of insects and plants respectively), 2) the number of realized links (the actual number of trophic interactions that occur between the species in the network), 3) linkage density (the mean number of links per species), and 4) connectance (the proportion of the total number of possible interactions between species that actually occur, calculated by dividing the number of maximum links possible by the number of realized links; resulting in a value bound between 0 and 1).

Q2) Have the flower and insect visitor communities been reinstated in restored heathlands?

The flower and visitor communities were compared between restored and ancient heathland of the pairs for both 2001 and 2004. Parameters compared for the floral community included floral abundance, species richness, the proportional distribution of the dominant floral species, species diversity (using the Shannon Weaver Index), and community evenness (where a value of 1 indicates the equal representation of each species in the community) (Magurran 2004). Parameters compared for the insect community included the abundance and species richness of all insect species.

of Hymenoptera, of Diptera, and of the remaining species. Species diversity and community evenness values were also calculated for the whole insect community.

All analyses were carried out using Minitab version 14.13 (Minitab Inc. 2004), and proportional data were arcsine-root transformed prior to analysis. Paired T-tests and its non-parametric equivalent the Wilcoxon signed rank test were used to compare restored communities to their paired ancient communities for these community parameters. This was done within each of the two years these sites were sampled. Communities sampled from restored and ancient sites in 2001 were further compared to their respective communities sampled again in 2004 to determine the extent to which they had changed over time.

Q3) Do the pollinators of restored heaths originate from their adjacent, ancient heaths?

The percentage of insect species shared between any two sites sampled was used as a measure of similarity in species composition and calculated using the raw data on species richness collected for each site. This presence / absence measure was further complemented by re-calculating the percentage of species overlap after weighting for abundance. In this way the percentage of the total visitor abundance represented by shared species of any two sites was calculated.

Q4) Did the pollinators of restored heaths become more similar in composition to ancient heaths over time?

Species composition at restored and ancient heaths were compared between 2001 and 2004 using Paired T-tests. Correspondence analysis, an ordination technique, was also used to graphically describe the variation in the species composition over time.

2.3 Results

Q1) Are the interaction networks of ancient heaths more complex than those of restored heathlands?

The plant and insect communities of restored and ancient heathland sites sampled were linked into complex visitation networks, with entomophilous insects linked to floral species through their foraging visits. Such plant-visitor interactions can be illustrated quantitatively through interaction networks that describe both the abundance and species richness of the two trophic levels involved. Figure 2.2 provides an illustration of an interaction network for one of the four pairs of restored and ancient heathland sampled in 2004 (see Appendix A for remaining networks).

Network statistics were calculated for each community for both 2001 and 2004. In 2001 the interaction networks of ancient heathland sites had significantly greater numbers of realized links than did their paired restored sites, leading to significantly higher linkage density and connectance values (Table 2.2). By 2004, the number of realized links between restored and ancient heaths were no longer different, although both linkage density and connectance values were still significantly greater at the ancient sites of the pairs (Table 2.3). Also, ancient community networks significantly increased in their connectance between 2001 and 2004 (Paired T-test, $t = -6.79$, $p = 0.007$). The increase in connectance found for restored communities between 2001 and 2004 however were of marginal significance (Paired T-test, $t = -3.19$, $p = 0.050$) (Table 2.4 and 2.5).

Q2) Have the flower and insect visitor communities been reinstated in restored heathlands?

The floral community: In both 2001 and 2004, the floral community of the heathlands sampled were low in species diversity, a typical characteristic of heathlands. Communities were dominated by two species of Ericaceae, *Erica cinerea* and *Calluna vulgaris*. Other core flowering species found in all plots included the

ericaceous species *Erica tetralix,* and three species of Fabaceae. *Ulex europaeus*, *Ulex minor*, and *Ulex gallii*. A further Red Data Book species confined solely to the Dorset heathlands, *Erica cilliaris*, was recorded in R1 in 2004 only. No significant differences were found in the abundance of floral resources available to foraging insects between restored and ancient sites, in either 2001 or 2004 (Table 2.2 and 2.3).

In 2001, ancient sites had significantly greater flowering proportions of *Erica spp.* (*Erica cinerea* and *Erica tetralix* combined) and of *E. cinerea* than did the restored sites of the pairs, although this significance was lost by 2004 (Table 2.2). Restored sites however had significantly greater flowering proportions of *C. vulgaris* than did ancient sites in both 2001 and 2004 (Table 2.2 and 2.3). Between 2001 and 2004, floral species richness decreased at restored sites (Table 2.4). This was mainly accounted for by the reduction in the number of ruderal, weedy species recorded at low abundance in restored sites in 2001, and was further reflected in the significant reduction in floral species diversity calculated for each sampling year (Table 2.4). The floral community at restored sites also significantly increased in evenness over time (Table 2.4). Ancient sites however showed no change in its floral community between 2001 and 2004 (Table 2.5).

The insect community: In 2001, a total of 2723 insect visitors represented by 112 identified species were sampled from the four pairs of restored and ancient dry lowland heathland sites over the season. 58.87% of visitors were sampled from the four ancient heathland sites, with 41.13% from the restored sites of the pairs. In 2004, a total of 2836 insect visitors represented by 126 identified species were sampled. 51.26% of these visitors were sampled from the four ancient heathland sites, with 48.74% from the restored sites of the pairs (Appendix A).

In 2001, ancient heaths had significantly greater species richness than restored heaths (Table 2.2), although no differences were found between ancient and restored sites by 2004 (Table 2.3). All other insect community parameters measured did not differ between restored and ancient communities for either year sampled. Similarly, measures of diversity and evenness of the insect community did not differ between pairs of restored and ancient sites in either 2001 or 2004 (Table 2.2 and 2.3). However between 2001 and 2004, restored communities significantly increased in

dipteran abundance (Table 2.4), whilst ancient communities increased in evenness (Table 2.5). All other parameters measured showed no change over time.

Q3) Do the pollinators of restored heaths originate from their adjacent, ancient heaths?

Whilst Figure 2.3 shows a correlation in insect species richness between paired restored and ancient heathland communities, there was little evidence to suggest the origin of species at restored sites from their adjacent, ancient heathland sites. In 2001, paired restored and ancient sites shared only between 26.67% and 36.92% of their species, although the restored sites of pairs 2, 3 and 4 shared more species with their respective ancient site than any other site (Table 2.6a). However the increased similarity within these three pairs was very slight. Furthermore, the greatest overlap in species composition was still only 37.04% of the total number of species (between sites A2 and A4). In 2004, paired sites shared a slightly greater proportion of species than in 2001, ranging between 30.30% and 41.86% of species shared, but only in pairs 1 and 4 did the restored site have the greatest overlap with its respective ancient site than with any other site (Table 2.6b). The greatest overlap in species composition however was still only 45.65% (between A2 and A3).

When the proportions of species overlap were weighted in terms of their abundance, pairs of restored and ancient sites showed much greater overlaps in their species composition. In 2001, between 86.12% and 93.86% of visitors were from shared species, with pairs 1 and 4 showing the greatest overlap within each other than with other sites (Table 2.6a). Such high percentage abundances of overlap in both years can be attributed to the high proportion of honeybees that dominated the insect community, followed by various species of bumblebees, particularly *B. lucorum* and *B. terrestris*. In 2004, between 88.55% and 92.66% of visitors were from shared species, with restored sites in pairs 3 and 4 showing the greatest overlap within each other than with other sites (Table 2.6b). Again this could be attributed to the dominance of honeybees and bumblebees at the heathland sites.

Q4) Did the pollinators of restored heaths become more similar in composition to ancient heaths over time?

The percentage of species overlap in the insect communities of restored and ancient sites increased significantly from 2001 to 2004 (Paired T-test, $t = -4.35$, $p = 0.001$). However the mean proportion of species common to both restored and ancient sites in 2004 were still relatively low, at 32.11 ± 1.25% (compared to 27.97 ± 1.08% of species shared between restored and ancient sites in 2001). When the data were weighted in terms of species abundance, no differences were found in the proportion of overlap between restored and ancient communities over time (Paired T-test, $t = 0.09$, $p = 0.931$).

However it was evident that the insect community composition varied dramatically over time. When comparing the percentage of species common to both years for any given site, the percentage of overlap ranged between only 21.21% and 39.02% (Table 2.7), with the remaining 61% to 79% of the insect community changing in composition at the same site between 2001 and 2004. Yet after weighting for abundance, a much greater overlap was seen, with the identity of between 75.48% and 92.15% of visitors being similar between 2001 and 2004. Thus a minority of insect visitor species were consistent between years, with the majority fluctuating over time. To illustrate this temporal variation in species assemblages between restored and ancient sites, the first two dimensions of a correspondence analysis are presented in Figure 2.4. Each point represents the species composition for a site sampled over a whole season for each year it was sampled. Immediately apparent is the separation of sites into clusters according to year along the first component axis (dominant over the 2nd axis), with no pattern emerging for site treatment (i.e. restored or ancient sites). Annual stochasticity is therefore an important factor determining species assemblages across the heathland.

2.4 Discussion

This study found the floral and insect communities successfully reinstated at restored heathland, following fourteen years of restorative management. However measures of functional complexity of the plant-visitor networks remained reduced at restored sites compared to their paired ancient reference networks, whilst differences in floral composition were still present between restored and ancient sites. With regard to species composition, adjacent heathlands did not share any more species than with more distant heaths, suggesting that the requirement for restoration sites to be located adjacent to ancient communities is unnecessary, with regard to the re-establishment of insect visitors. Furthermore, a large proportion of species were found to vary markedly over both time and space, with most of this variation attributable to sampling year. However the great majority of visitors to heaths, represented by only a relatively small proportion of species, were consistent over both space and time, suggesting the successful reinstatement of the core pollinator guild to restored heathlands.

The network approach

Rather than determining measures of community structure alone such as species abundance and composition, this study also analyses the interactions between species, allowing a more informed approach to evaluating community restoration. By mapping species interactions into community networks, the networks for pairs of restored and reference heathlands could be compared, providing an indication for the reinstatement of pollinator interactions. Furthermore this study is unique to restoration ecology not only by taking a network approach in evaluating restoration success, but also by using a sampling protocol with replication in both time and space. Such replication is essential to enable true differentiation between natural variation and variation due to the restorative treatment itself (White & Walker 1997), yet replication is a rare approach in ecological restoration particularly at the community level. This is typically due to the economic limitations of most restoration programmes, combined with the complexity of communities and

limitations in obtaining adequate or appropriate reference sites (Michener 1997; Palmer *et al.* 1997; White & Walker 1997; Ruiz-Jaen & Aide 2005).

Limitations

This study was limited to four replicate pairs of ancient and restored heathland. Although this is one of few restoration studies that are replicated in both space and time, a greater number of spatial replicates would have been beneficial to determine the extent of variation of both reference and restored heathland communities. Knowing the degree of community variation at reference sites is important in the evaluation of restoration success, as acceptable levels of variation at restored sites can then be determined. In Chapter 3, I extend this paired design to six pairs of reference and restored sites.

A further limitation of this study is its focus on the pollinator community. The insect community studied here consisted of 'potential' pollinators. By this I refer to those visiting insects that exploit floral resources, and in doing so, pick up, transfer and deposit pollen upon their next visit. However insects vary in the quantity and quality of con-specific pollen they carry and transfer, thereby varying in the quality of their pollination service, if offering one at all (Tepedino 1981; Herrera 1987; Stanton *et al.* 1991; Wilson & Thomson 1991). Yet it remains necessary to first evaluate whether the species assemblage and their interactions have been successfully reinstated, before an attempt can be made to determine whether pollination itself can return to the restored community (Thompson & Pellmyr 1992; Williams *et al.* 2001). Moreover, visitor abundance and the frequency of their interactions have been found to be effective surrogate measures in determining their contribution to plant reproductive success (Morris 2003; Vazquez *et al.* 2005). In this study, the reinstatement of interactions between insect visitors and their floral resources are therefore used simply as an indication, rather than a determinant, of returning pollinator function to restored systems.

Q1) Are the interaction networks of ancient heaths more complex than restored heaths?

Despite no significant differences in the size of the heathland community networks between sites or between sampling years, the density of interactions and the proportion of the total possible interactions actually realized between species, i.e. the connectance of the networks, were greater in ancient networks. Ancient heathlands presented well-established floral resources that varied less in their availability than at restored sites, thereby providing a more reliable resource required by their visiting pollinators over time. This greater variability in resources at restored sites may have reduced the potential for the full establishment of interactions between plants and their insect visitors, reflected by the lower connectance of their plant-visitor networks.

Such network properties can determine the robustness of a community to both primary and secondary species extinctions (Dunne *et al.* 2002; Ives & Cardinale 2004; Memmott *et al.* 2004; Sole & Montoya 2006). For example, a study by Dunne *et al.* (2002), analysing the connectance of 16 food webs and their tolerance to extinctions, found a significant positive relationship between connectance and robustness towards extinctions. However the connectance values of their webs analysed differed by ten-fold. In this study, the connectance values of the ancient community networks were no greater than 1.4 times that of restored community networks in both sampling years. Thus although differences still persisted between site types, the lag of restored sites behind ancient sites, with regards to their return of pollinator interactions and network complexity, may in reality be insufficient to substantially affect the resilience of restored communities to environmental perturbations. Furthermore, using the same modelling techniques of Dunne *et al.* (2002), Forup *et al.* (*in press*) found no significant differences in the robustness between ancient and restored networks when simulating species extinctions for the heathland networks described in this study (where 'robustness' was quantified by determining the proportion of species that had to be removed before a loss of ≥ 50% of species (considering both primary and secondary extinctions) could be initiated).

Q2) Have the flower and insect visitor communities been reinstated in restored heathlands?

Despite differences in the interaction networks between ancient and restored sites, the majority of measures of community structure did not differ between restored and ancient heathlands, with both the floral and insect communities successfully reinstated. However differences in the proportional abundance of *Erica spp.* and *C. vulgaris,* the two dominant heathland plant species, remained between ancient and restored heathlands after eleven (2001) and fourteen (2004) years of restorative management. *C. vulgaris* was found to dominate restored sites, with 71% of the total floral resources available accounted for by this species. This can be explained by the life-cycle phases of *C. vulgaris.* At twelve and thirteen years of age, *C. vulgaris* reaches the end of its 'building' phase and the start of its 'mature' phase, following its initial 'pioneer' period which would have begun at the onset of restoration of these formerly afforested sites (Barclay-Estrup 1971). During the building and mature phases of *C. vulgaris*, net production of its shoots reach its maximum, resulting in *C. vulgaris* typically contributing to between 75% and 85% of total heathland coverage (Barclay-Estrup & Gimingham 1969; Barclay-Estrup 1970), as seen in the restored heathlands studied here. At twenty-nine or more years of age however, *C. vulgaris* begins its 'degenerative' phase, where it contributes to only up to 35% of heathland cover (Barclay-Estrup 1971); a situation reflected at ancient sites where the proportion floral resources offered by *C. vulgaris* was only 42% in 2001 and 37% by 2004, being dominated instead by *Erica spp.*.

As a consequence of differences in proportional composition, ancient sites flowered much earlier than their respective restored sites, with *Erica* species beginning their flowering in early June and peaking between late July and early August. The flowering phenology of *C. vulgaris* instead lagged behind, beginning only in late July and peaking by mid to late August. However despite this lag in floral resources at restored heaths, only in 2001 were differences found in their insect communities, with greater numbers of visiting species sampled at ancient heaths. However this difference was no longer found by 2004. Thus differences in floral resource availability, still present following fourteen years of restorative management, appeared to have minimal impact upon the insect visitor communities.

Q3) Do the pollinators of restored heaths originate from their adjacent, ancient heaths?

Usually restored heathlands in Dorset are situated close to ancient heathland fragments. Consequently I expected that the insect species re-establishing on restored sites would be predominantly from these adjacent ancient heathlands. Although restored sites of three of the four pairs (pairs 2, 3, and 4) in 2001 had greater similarities in their species composition with their paired ancient site than with more distant ancient sites, the actual proportions of species shared between the pairs was consistently low at a maximum of 36.92%. Furthermore, in 2004 this was only the case for pairs 1 and 4, with only a maximum of 41.86% of species shared. Such a low proportion of species common between pairs of sites most likely reflects the spatiotemporal variability of insect populations found more generally in plant-pollinator systems (Herrera 1988; Fishbein & Venable 1996; Roubik 2001; Price *et al.* 2005), whereby the large proportion of rarer species occur in local species pools simply by chance events.

However when weighting for the abundance of visiting species, over 86.12% in 2001 and over 88.15% in 2004 of visitors represented species shared between sites of the pairs. This highlight the dominance in abundance by a relatively small proportion of species, mostly accounted for by *Apis mellifera* and *Bombus* species (particularly *Bombus lucorum* and *B. terrestris*), followed by dipteran species from the family Syrphidae, consistent over both time (two years) and space (8 sites). Furthermore, these super-abundant core species are highly mobile, suggesting that heathland restoration schemes do not necessarily have to exist adjacent to reference sites for their insect communities to return. This situation supports the "core-satellite" hypothesis (Hanski 1982; Gotelli & Simberloff 1987), which predicts a bimodal distribution of regional species at local patches, whereby a local patch is expected to be dominated in abundance by a low number of "core" species occurring in high abundance within the local patch, followed by a larger number of "satellite" species that occur at much lower abundances. This distribution is typically due to the better colonisation abilities of "core" species relative to "satellite" species, leaving the latter more susceptible to extinction from both local and regional pools. In the context of restoration, identifying "core" over "satellite" species is critical in

promoting the reinstatement of ecological function, and indeed the presence of core species at restored sites suggest that the return of function, with regard to pollination, is likely close to full reinstatement.

Q4) Did the pollinators of restored heaths become more similar in composition to ancient heaths over time?

Between 2001 and 2004 restored sites significantly increased in the number of insect species shared with ancient sites. This increase over time may suggest that the reinstatement of insect communities to their full complement at restored sites, as determined by their reference ancient sites, may still be occurring following fourteen years of restorative management. However the extent to which the species pools at reference heaths can determine species composition at restored communities remains unknown. Indeed the species composition of pollinator visitors to heaths was highly variable between sites, with the majority of species fluctuated greatly over both time and space, the former attributing the greatest to this variation in insect community composition.

Conclusions

Ecological restoration can potentially speed the recovery of degraded ecosystems through the provision of favourable conditions for species re-establishment. However restoration also has the potential to encourage interactions to rebuild between species, thereby reinstating the essential ecological processes and services which infer function to a community (Dobson *et al.* 1997; Palmer *et al.* 1997; Neal 1998; Young 2000). By taking a network approach in evaluating restoration success, this study emphasizes the importance of recognizing the interactions between species, rather than the species themselves, as the key drivers that sustain and regulate ecological communities (Memmott *et al.* 2006; Zanden *et al.* 2006; Forup *et al. in press*).

2.5 Tables & Figures

Table 2.1 Twelve heathland sites located in the Purbeck District, South Dorset. Four pairs of ancient (A1-4) and restored (R1-4) sites and four additional ancient (A5-8) heathland sites are listed, together with their management authority, grid reference, area, metres above sea level. 'Heath area' is the area of heathland coverage upon which the sampling plot was established. Areas in () refer to the area of the whole site and not of the heathland alone, as this had not yet established by the management body. All sites are extant fragments of the once extensive Dorset heathland matrix (6000ha).

		Study site	Locality	Management Body	Grid reference		Heath Area (ha)	metres asl.
					SY / SZ	BNG		
Pair 1	R1	Arne restored	Grip Heath	RSPB	SY 97349	87747	8	7
	A1	Arne old	Grip Heath	RSPB	SY 97386	87595	30	5
Pair 2	R2	Holton restored	Holton Lee	Holton Lee Charity / RSPB	SY 95927	91775	15	0
	A2	Gore old	Great Ovens	Herpertological Cons. Trust	SY 92616	90141	25	19
Pair 3	R3	Hyde restored	Lower Hyde	Forestry Commission	SY 87868	90993	2	35
	A3	Hyde old	Lower Hyde	Private landowner	SY 88321	91053	18	25
Pair 4	R4	Morden restored	Morden Bog	English Nature	SY 90898	92235	8	15
	A4	Morden old	Morden Bog	English Nature	SY 91143	92235	16	15

Table 2.2 Comparing four restored heathland communities (R sites) to their paired ancient heathland communities (A sites) for community parameters measured in 2001 using Paired T-tests. Significant p values (quoted at 95% confidence levels) are indicated by * ($p < 0.05$) and ** ($p < 0.005$).

2001 data	Variable	Mean for A sites	± Standard Error	Mean for R sites	± Standard Error	Paired Test	Test Statistic	p value	Sig.
Floral parameters									
	Floral abundance	13124.10	2270.30	10891.90	608.00	Paired T-test	0.90	0.437	
	Floral species richness	6.00	0.41	8.00	0.82	Paired T-test	-2.19	0.116	
	Proportion of *Erica spp.*	0.57	0.04	0.28	0.04	Paired T-test	6.19	0.008	*
	Proportion of *Erica cinerea*	0.48	0.04	0.26	0.03	Paired T-test	10.61	0.002	**
	Proportion of *Calluna vulgaris*	0.42	0.04	0.71	0.04	Paired T-test	-6.48	0.007	*
	Floral evenness	0.56	0.04	0.41	0.03	Paired T-test	2.76	0.070	
	Floral species diversity	0.95	0.06	0.75	0.07	Paired T-test	1.98	0.141	
Visitor parameters									
	Insect abundance	400.75	25.72	280.00	41.31	Paired T-test	1.83	0.164	
	Insect species richness	39.25	3.77	32.50	4.21	Paired T-test	4.36	0.022	*
	Hymenopteran abundance	312.50	28.96	88.05	44.03	Paired T-test	1.59	0.211	
	Hymenopteran species richness	14.25	0.63	10.25	1.11	Paired T-test	2.53	0.085	
	Dipteran abundance	79.00	18.36	51.75	9.37	Paired T-test	1.64	0.200	
	Dipteran species richness	21.00	3.89	18.25	2.29	Paired T-test	1.29	0.288	
	Other species abundance	9.25	3.35	10.25	3.94	Paired T-test	-0.23	0.834	
	Other species richness	4.00	0.41	4.00	1.41	Paired T-test	0.00	1.000	
	Insect evenness	0.56	0.06	0.54	0.08	Paired T-test	0.33	0.762	
	Insect species diversity	2.07	0.25	1.88	0.33	Paired T-test	0.84	0.461	
Network statistics									
	Maximum links possible	237.75	33.18	266.50	56.65	Paired T-test	-0.90	0.436	
	Realized links	61.75	6.86	46.50	6.51	Paired T-test	9.23	0.003	**
	Linkage density	1.35	0.05	1.13	0.04	Paired T-test	4.55	0.020	*
	Connectance	0.26	0.01	0.18	0.02	Paired T-test	5.06	0.015	*

Table 2.3 Comparing four restored heathland communities (R sites) to their paired ancient heathland communities (A sites) for community parameters measured in 2004 using Paired T-tests and its non-parametric equivalent, the Wilcoxon Signed-Rank test. Significant p values (quoted at 95% confidence levels) are indicated by * ($p < 0.05$) and ** ($p < 0.005$).

2004 data	Variable	Mean for A sites	Standard Error	Mean for R sites	Standard Error	Paired Test	Test Statistic	p value	Sig.
Floral parameters									
	Floral abundance	2097.11	142.26	2341.85	227.73	Paired T-test	-0.90	0.435	
	Floral species richness	4.50	0.50	5.50	0.65	Paired T-test	-1.41	0.252	
	Proportion of *Erica spp.*	0.62	0.07	0.26	0.04	Wilcoxon signed rank	10.00	0.100	
	Proportion of *Erica cinerea*	0.52	0.08	0.25	0.04	Wilcoxon signed rank	10.00	0.100	
	Proportion of *Calluna vulgaris*	0.37	0.07	0.71	0.05	Paired T-test	-3.74	0.033	*
	Floral evenness	0.60	0.08	0.46	0.03	Paired T-test	1.86	0.159	
	Floral species diversity	0.89	0.11	0.70	0.07	Paired T-test	2.18	0.117	
Visitor parameters									
	Insect abundance	362.25	60.16	344.75	66.05	Paired T-test	0.41	0.711	
	Insect species richness	32.00	2.48	40.50	4.65	Paired T-test	-2.59	0.081	
	Hymenopteran abundance	246.50	56.27	210.00	42.26	Paired T-test	0.71	0.531	
	Hymenopteran species richness	13.25	0.85	13.00	0.41	Paired T-test	0.21	0.846	
	Dipteran abundance	113.00	25.80	132.75	27.86	Paired T-test	-0.70	0.534	
	Dipteran species richness	17.50	2.33	25.25	4.11	Paired T-test	-2.51	0.087	
	Other species abundance	2.75	2.14	2.00	0.71	Wilcoxon signed rank	4.00	0.855	
	Other species richness	1.25	0.48	2.25	0.95	Paired T-test	-1.41	0.252	
	Insect evenness	0.66	0.05	0.68	0.04	Paired T-test	-0.63	0.571	
	Insect species diversity	2.29	0.20	2.4.95	0.13	Paired T-test	-1.34	0.274	
Network statistics									
	Maximum links possible	147.00	27.64	221.00	29.73	Paired T-test	-3.02	0.057	
	Realized links	51.00	5.92	56.50	6.93	Paired T-test	-1.31	0.283	
	Linkage density	1.39	0.06	1.22	0.05	Paired T-test	4.85	0.017	*
	Connectance	0.36	0.02	0.26	0.02	Paired T-test	4.03	0.028	*

Table 2.4 Comparing the community parameters measured for four restored heathland communities sampled in 2001 and 2004 using Paired T-tests. Significant p values (quoted at 95% confidence levels) are indicated by * ($p < 0.05$) and ** ($p < 0.005$).

Restored Sites	Variable	Mean for 2001	± Standard Error	Mean for 2004	± Standard Error	Paired Test	Test Statistic	p value	Sig.
Floral parameters									
	Floral species richness	8.00	0.82	5.50	0.65	Paired T-test	3.87	0.030	*
	Proportion of *Erica spp.*	0.28	0.04	0.26	0.04	Paired T-test	0.46	0.678	
	Proportion of *Erica cinerea*	0.26	0.03	0.25	0.04	Paired T-test	0.42	0.704	
	Proportion of *Calluna vulgaris*	0.71	0.04	0.71	0.05	Paired T-test	-0.39	0.723	
	Floral evenness	0.41	0.03	0.46	0.03	Paired T-test	-4.62	0.019	*
	Floral species diversity	0.75	0.07	0.70	0.07	Paired T-test	7.55	0.005	*
Visitor parameters									
	Insect abundance	280.00	41.31	344.75	66.05	Paired T-test	-1.73	0.182	
	Insect species richness	32.50	4.21	40.50	4.65	Paired T-test	-1.17	0.325	
	Hymenopteran abundance	218.00	44.03	210.00	42.26	Paired T-test	0.34	0.754	
	Hymenopteran species richness	10.25	1.11	13.00	0.41	Paired T-test	-3.67	0.035	*
	Dipteran abundance	51.75	9.37	132.75	27.86	Paired T-test	-2.44	0.093	
	Dipteran species richness	18.35	2.29	25.25	4.11	Paired T-test	-1.31	0.281	
	Other species abundance	10.25	3.94	2.00	0.71	Paired T-test	1.83	0.165	
	Other species richness	4.00	1.41	2.25	0.95	Paired T-test	0.85	0.457	
	Insect evenness	0.54	0.08	0.68	0.04	Paired T-test	-2.69	0.074	
	Insect species diversity	1.88	0.33	2.50	0.29	Paired T-test	-2.10	0.127	
Network statistics									
	Maximum links possible	266.50	56.65	221.00	29.73	Paired T-test	0.80	0.482	
	Realized links	46.50	6.51	56.50	6.93	Paired T-test	-1.03	0.380	
	Linkage density	1.13	0.04	1.22	0.05	Paired T-test	-1.15	0.333	
	Connectance	0.18	0.02	0.26	0.02	Paired T-test	-3.19	0.050	

Table 2.5 Comparing the community parameters measured for four ancient heathland communities sampled in 2001 and 2004 using Paired T-tests and its non-parametric equivalent, the Wilcoxon Signed-Rank test. Significant p values (quoted at 95% confidence levels) are indicated by * ($p < 0.05$) and ** ($p < 0.005$).

Ancient Sites	Variable	Mean for 2001	Standard Error	Mean for 2004	Standard Error	Paired Test	Test Statistic	p value	Sig.
Floral parameters									
	Floral species richness	6.00	0.41	4.50	0.50	Wilcoxon signed rank	10.00	0.100	
	Proportion of *Erica spp.*	0.57	0.04	0.62	0.07	Paired T-test	-0.51	0.648	
	Proportion of *Erica cinerea*	0.48	0.04	0.52	0.08	Paired T-test	-0.48	0.661	
	Proportion of *Calluna vulgaris*	0.42	0.04	0.37	0.07	Paired T-test	0.51	0.644	
	Floral evenness	0.56	0.04	0.60	0.08	Paired T-test	-0.60	0.592	
	Floral species diversity	0.95	0.06	0.89	0.11	Paired T-test	0.61	0.586	
Visitor parameters									
	Insect abundance	400.75	25.72	362.25	60.16	Paired T-test	0.60	0.591	
	Insect species richness	39.25	3.77	32.00	2.48	Paired T-test	1.66	0.195	
	Hymenopteran abundance	312.50	28.96	246.50	56.27	Paired T-test	1.89	0.154	
	Hymenopteran species richness	14.25	0.63	13.25	0.85	Paired T-test	0.93	0.423	
	Dipteran abundance	79.00	18.36	113.00	25.80	Paired T-test	-1.12	0.340	
	Dipteran species richness	21.00	3.89	17.50	2.33	Paired T-test	0.89	0.438	
	Other species abundance	9.25	3.35	2.75	2.14	Paired T-test	1.48	0.237	
	Other species richness	4.00	0.41	1.25	0.48	Wilcoxon signed rank	10.00	0.100	
	Insect evenness	0.56	0.06	0.66	0.05	Paired T-test	-4.50	0.021	*
	Insect species diversity	2.07	0.25	2.29	0.20	Paired T-test	-1.53	0.223	
Network statistics									
	Maximum links possible	237.75	33.18	147.00	27.64	Wilcoxon signed rank	10.00	1.000	
	Realized links	61.75	6.86	51.00	11.83	Wilcoxon signed rank	10.00	1.000	
	Linkage density	1.35	0.05	1.39	0.07	Paired T-test	-0.65	0.562	
	Connectance	0.26	0.01	0.36	0.02	Paired T-test	-6.79	0.007	*

Table 2.6 Percentage similarity in insect species composition between four pairs (1-4) of ancient (A) and restored (R) sites sampled in a) 2001 and b) 2004. Unshaded values refer to species that are common to both sites as a percentage of the total number of species found at both sites. Shaded values refer to the total abundance of individuals that are from species common to both sites as a percentage of the total number of individuals found at both sites.

a)

2001	A1	A2	A3	A4	R1	R2	R3	R4
A1	100.00	88.35	86.44	86.23	93.86	94.49	86.44	84.32
A2	31.03	100.00	88.47	93.45	85.84	89.43	87.76	90.39
A3	20.59	34.33	100.00	90.04	82.49	88.11	86.12	86.80
A4	26.92	37.04	29.03	100.00	87.03	93.03	88.31	93.59
R1	26.67	30.00	24.56	30.95	100.00	89.89	83.45	87.92
R2	26.32	31.15	28.36	29.63	27.08	100.00	88.51	90.47
R3	22.95	29.69	36.92	30.36	30.61	36.21	100.00	85.79
R4	17.74	27.27	24.59	30.43	27.50	24.53	27.78	100.00

b)

2004	A1	A2	A3	A4	R1	R2	R3	R4
A1	100.00	91.38	92.75	92.77	92.66	86.81	89.37	80.61
A2	32.65	100.00	90.31	91.99	86.99	90.14	84.33	87.75
A3	43.59	45.65	100.00	92.27	91.89	92.17	90.17	85.60
A4	38.64	44.00	37.21	100.00	91.98	90.72	87.98	88.55
R1	32.61	28.57	36.96	33.33	100.00	88.15	86.35	85.66
R2	26.79	34.43	37.74	32.20	21.88	100.00	92.41	88.20
R3	31.11	32.08	41.86	34.69	24.53	38.18	100.00	82.42
R4	19.70	28.57	32.79	30.30	26.47	35.21	27.27	100.00

Table 2.7 Percentage similarity in insect species composition for a site between 2001 and 2004 for four pairs (1-4) of ancient (A) and restored (R) sites. Unshaded values refer to species that are common to both sites as a percentage of the total number of species found at both sites. Shaded values refer to the total abundance of individuals that are from species common to both sites as a percentage of the total number of individuals found at both sites.

	A1	A2	A3	A4	R1	R2	R3	R4
% of species shared	27.08	32.79	30.51	34.69	39.02	22.39	23.73	21.21
% abundance of shared species	90.98	88.76	82.95	90.60	92.15	84.18	75.48	79.10

Figure 2.1 Purbeck District, located in the Poole Basin, south Dorset. The distribution of the four pairs of restored and ancient heathland sites are shown (•).

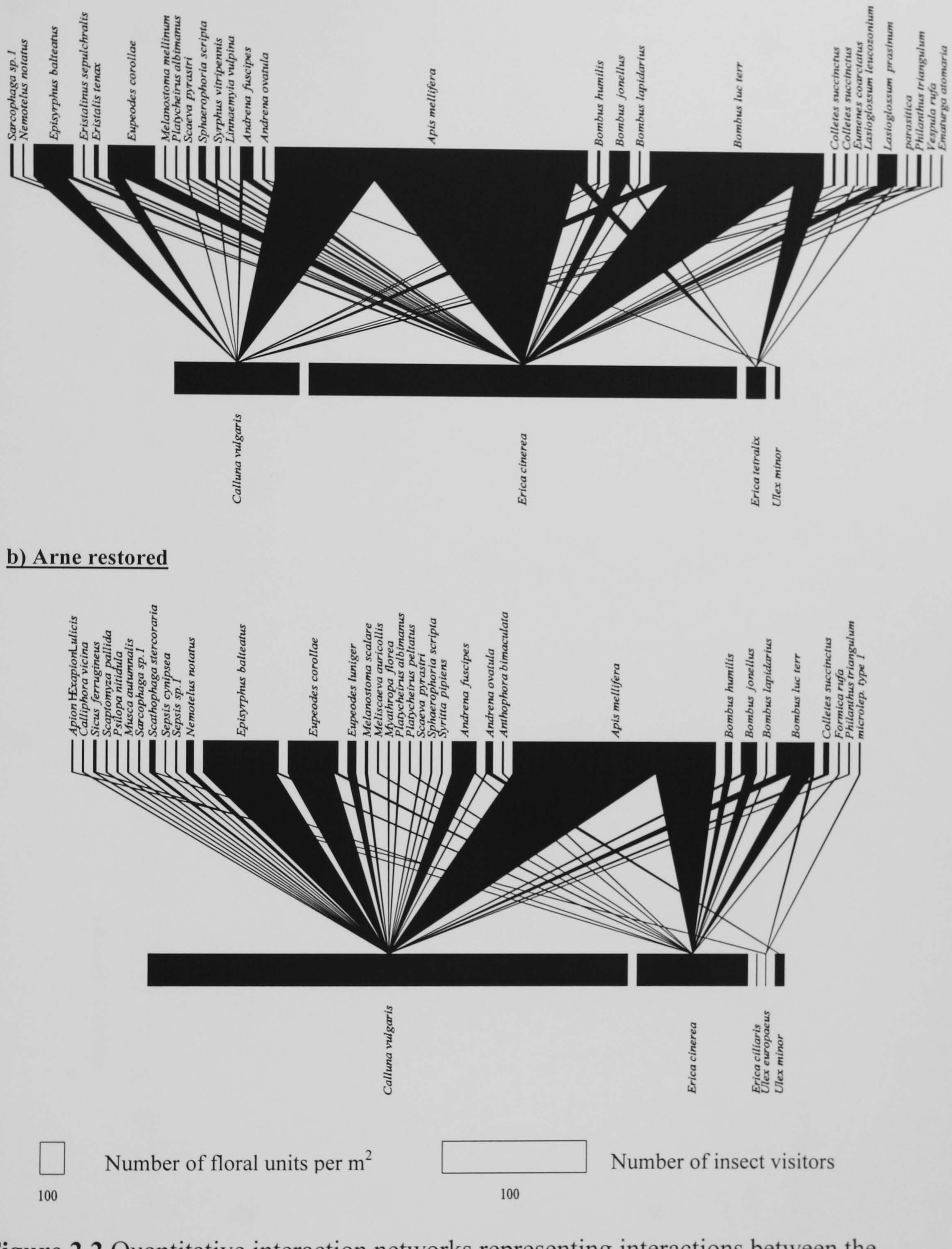
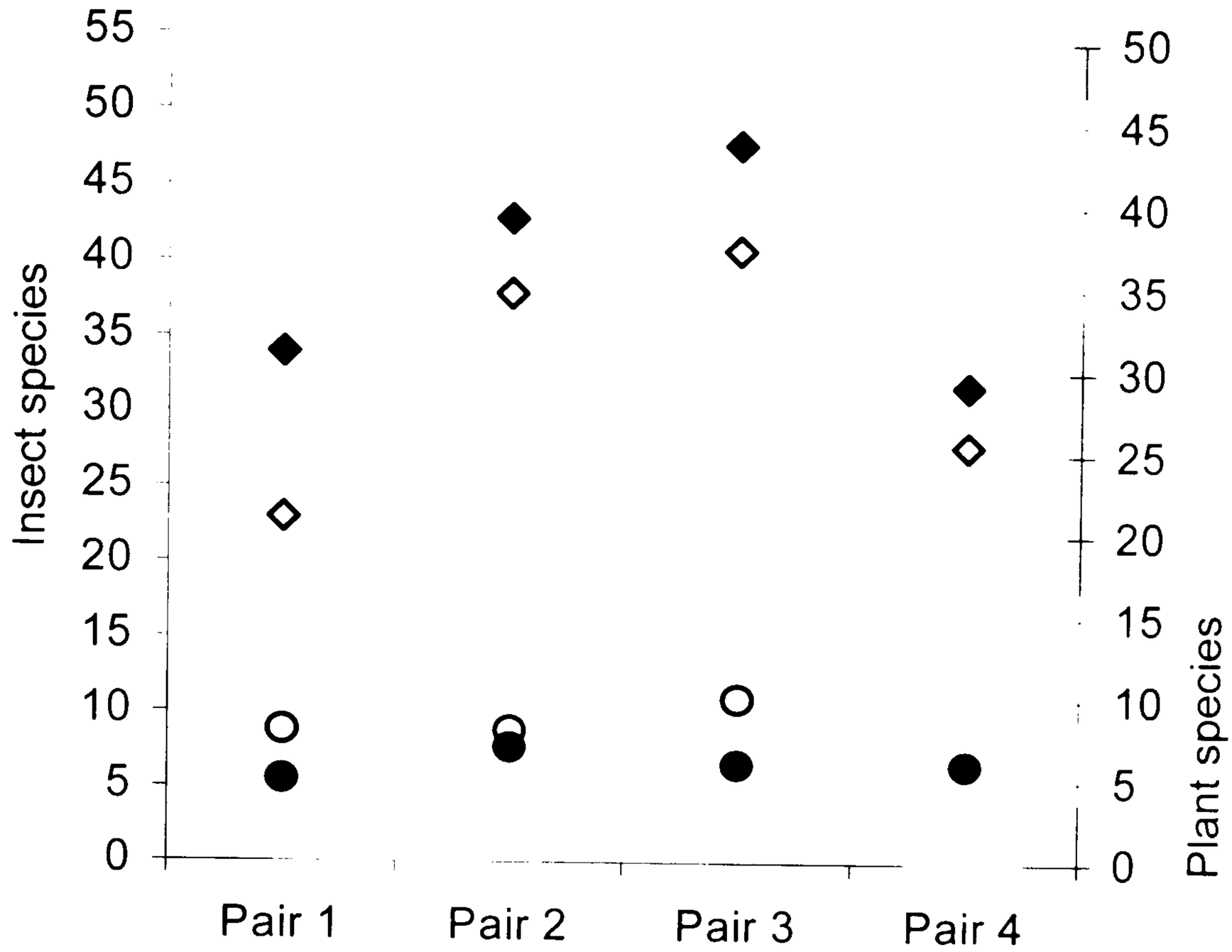


Figure 2.2 Quantitative interaction networks representing interactions between the heathland plant community and their insect visitors for a) ancient and b) restored heathland at Arne (Pair 1), sampled in 2004. The bottom row of rectangular bars represents the flower species whilst the upper row represents the insect visitors. Each rectangular bar in the rows represents a single species, their relative abundance being proportional to bar width. The scale bar at the base of the figure represents 100 floral units per m^2 of heathland and 100 individuals of visiting insects. The solid triangles that connect the two trophic levels represent the interactions linking the plant and insect species, the size of the triangle being proportional to the frequency of the interaction. Both webs are drawn to the same scale.

a)



b)

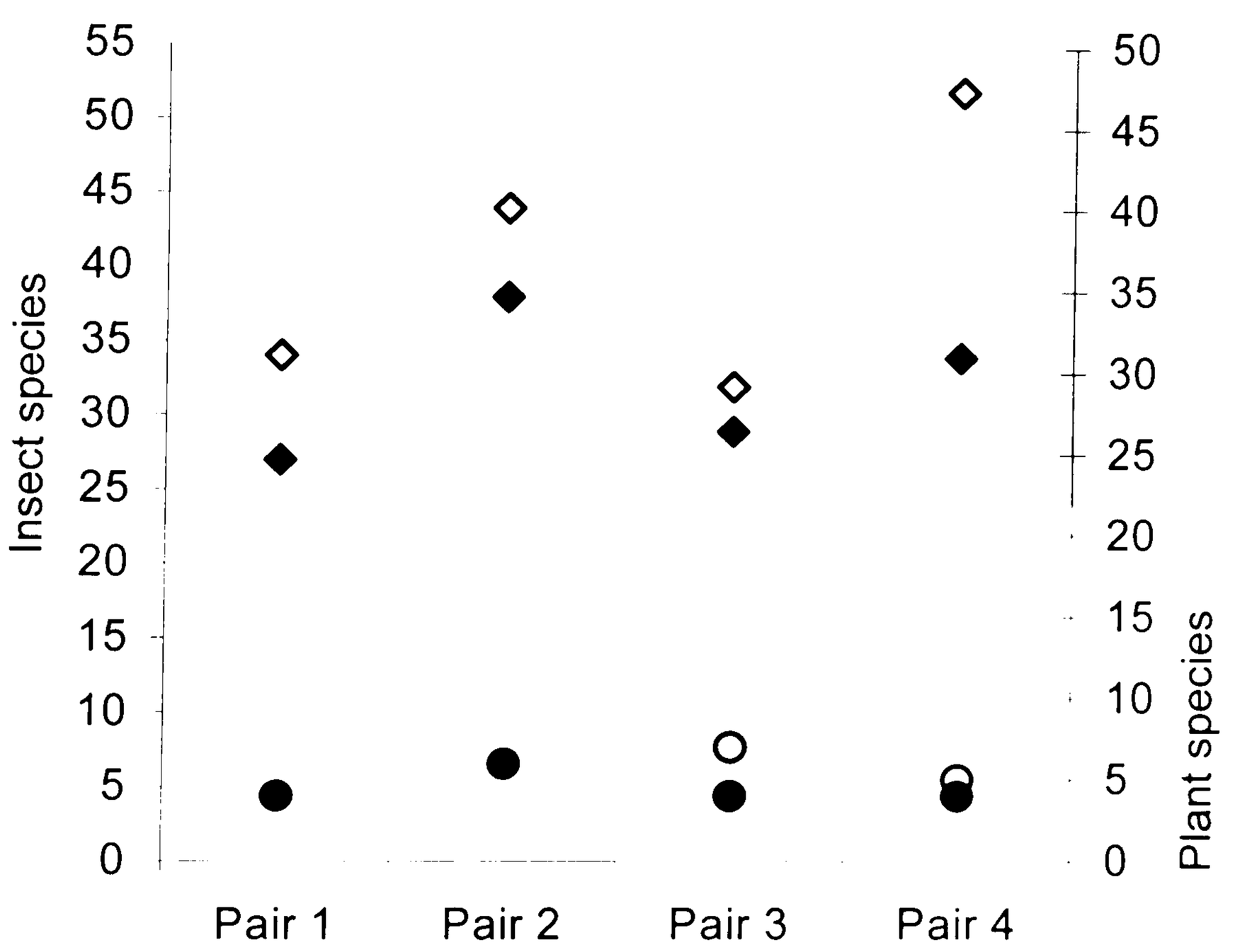


Figure 2.3 Plant and insect species richness on the restored and ancient heathlands in each of the four pairs in a) 2001 and b) 2004. ○ = plants, ◇ = insects, stippled lines = restored sites, filled lines = ancient sites.

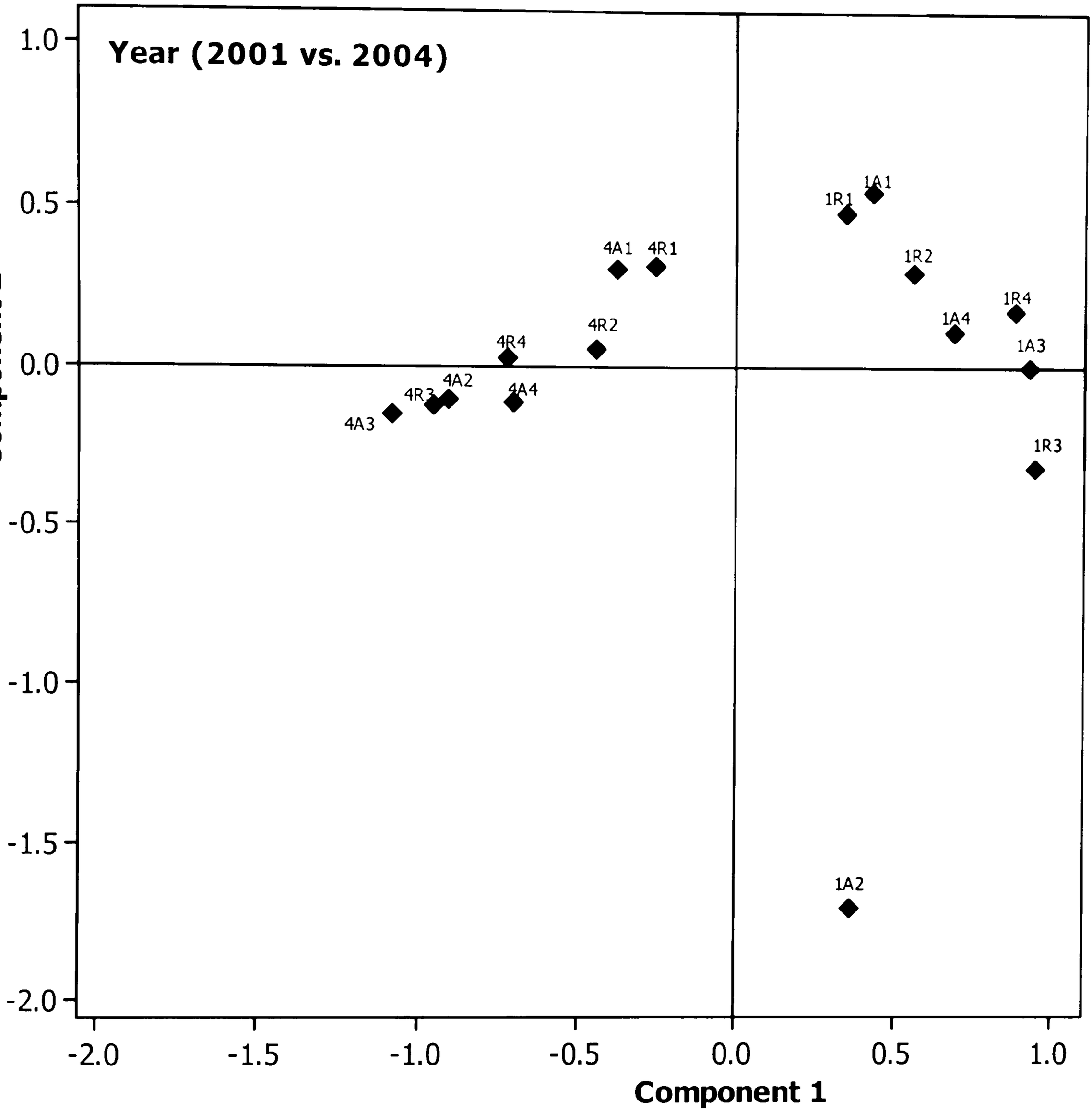


Figure 2.4 Correspondence analysis of insect species composition for four pairs of restored (R) and ancient (A) sites, sampled in 2001 and 2004. Site codes are labelled by the year sampled (1= 2001, 4=2004), then by site type (R or A) and finally by pair number (1-4).

CHAPTER THREE

Restoring parasites, parasitoids and pathogens: using the natural enemies of bumblebees as indicators of restoration success

In: **Henson, K.S.E.** & Memmott, J. Restoring an Ecological Network. *In prep.*

CHAPTER THREE

Restoring the parasites, parasitoids and pathogens: using the natural enemies of bumblebees as indicators of restoration success

Summary: *Interactions between species provide the 'glue' of biodiversity by linking them into communities, such that species exist in complex networks of interactions rather than in isolation. Using a matched pairs design, the assessment of restoration success of dry lowland heathland communities was extended to three trophic levels in 2005, incorporating 'hidden' links commonly ignored in ecological networks. Here, quantitative interaction webs were used to assess the degree to which interactions between flowering plants, their bumblebee pollinators and a phylogenetically diverse array of bumblebee parasites, parasitoids and pathogens were reinstated at restored relative to ancient reference sites.*

Comparing webs from six pairs of ancient and restored heathlands confirmed both plant and bumblebee communities to be successfully restored, together with four species of external phoretic mites and three internal gut protozoans. However, the re-establishment of one of the parasitoids, the dipteran family Conopidae, remained incomplete, with larval abundance, prevalence, frequency of conopid superparasitism, host load, and host range all significantly reduced at restored sites. Furthermore these differences were substantial enough to impact upon web topology measures, leading to significant differences in realized linkage between restored and ancient interaction networks.

This study supports the call for incorporating parasites into ecological networks, and highlights their particular worth in assessing restoration success, as their reliance upon the diversity and persistence of lower trophic levels will leave them to be amongst the last to re-establish in all ecological restoration projects.

3.1 Introduction

All species are linked into networks by direct and indirect interactions, which are collectively responsible for ecosystem processes and the provisioning of goods and services, such as pollination, seed dispersal, and pest control. With the value of ecological services provided by insects alone recently estimated at over 57 billion USD (Losey & Vaughan 2006), restoring a sustainable, functioning community is worthwhile from both ecological and economic perspectives (Balmford *et al.* 2002).

However the implications of habitat modification for ecosystem function are seldom fully recognised (Raffaelli *et al.* 2002; van der Putten *et al.* 2004; Tylianakis *et al.* 2007), as species interactions can be significantly altered without considerable impacts upon standard measures of species diversity or richness (Tylianakis *et al.* 2007). Changes in network topology (how species are linked together) for example, can affect the resilience of a community to both species loss (e.g. Memmott *et al.* 2004; Quince *et al.* 2005) and environmental perturbation (e.g. Ives & Cardinale 2004), whilst any re-arrangement of species interactions can impact upon compartments within the community network that may have initially been used to prioritise conservation efforts (Corbet 2000; Dicks *et al.* 2002).

Moreover the inclusion of "hidden" trophic levels such as host-parasite interactions can substantially affect measures of network stability (Lafferty *et al.* 2006), yet their incorporation into community studies continue to be ignored (Marcogliese & Cone 1997; Dobson *et al.* 2006). Indeed integrating research between trophic ecology and restoration ecology remains rare despite their potential to inform one another (Young *et al.* 2005). Furthermore by studying the trophic interactions within a community, large swathes of biodiversity often overlooked by conservation programmes can be revealed. Whilst the restoration of parasites may not be uppermost in the minds of conservation biologists, they remain key players in determining community structure and function (Dobson & Hudson 1986; Montoya *et al.* 2003) due to their ubiquitous nature, with almost 100% of all species are parasitized (Esch & Fernandez 1993).

With habitat degradation the greatest threat to biodiversity (Ewers & Didham 2006) and extinctions occurring at exponential rates (Raven 2002), theoretical models incorporating habitat destruction emphasise the likely build-up of an "extinction debt" of species, with payment due following further fragmentation and

isolation (Tilman *et al.* 1994). Restoration can delay this payment, and thus restoration ecology is considered a useful tool for investigating the mechanisms underlying the repair and recovery of degraded resources (Dobson *et al.* 1997; Palmer *et al.* 1997; Ormerod 2003). Such mechanisms include community assembly and the idea that species establishment can be sequentially dependent, such that species from higher trophic levels (e.g. herbivores, parasites) require both the presence and adequate abundance of lower-ranked trophic species (e.g. plants, hosts) to colonise successfully (Glasser 1982; Schoener 1989; Holt 1996). This dependence therefore leaves species from higher trophic levels differentially vulnerable to extinction, particularly in the case of small-bodied trophic specialists (e.g. parasites and parasitoids). Not only are these higher-ranked trophic specialists susceptible to impacts upon themselves, they are also affected by impacts to their lower-ranked trophic species (Didham *et al.* 1998; Holt *et al.* 1999). Furthermore, population size and densities of higher trophic level specialists are generally lower and more variable than species of lower trophic levels (Schoener & Spiller 1992; Kruess & Tscharntke 1994, 2000), and consequently their dispersal abilities are much reduced relative to those from lower levels (Kruess & Tscharntke 1994; Komonen *et al.* 2000).

In this study I use a food web approach to provide both a blueprint for restoration and a measure of restoration success. Working with three trophic levels on replicated pairs of restored and ancient heathlands in southern England, I constructed quantitative networks describing the interactions between flowering plants, bumblebee pollinators, and the parasites, parasitoids and pathogens of the bumblebees. I used species abundance and diversity of all three trophic levels as a measure of returning community structure, and the re-establishment of plant-pollinator and pollinator-parasite interactions as an indication of the reinstatement of community function. Specifically I hypothesise that 1) restoration of the lower trophic levels will be more complete than the upper trophic levels of the community as a result of greater susceptibility of higher-ranked species to demographic and environmental stochasticity, and 2) that measures of network topology will be affected as a consequence of altered species interactions, as incomplete reinstatement of species at higher trophic levels will impact upon the number of interactive links possible within the tri-trophic network.

3.2 Methods

Study system

Bumblebees (Hymenoptera: Apidae: *Bombus* spp.) are annual, eusocial insects with overlapping generations, and form an economically important pollinator guild that is declining throughout Europe and North America (reviewed in Goulson 2003). Bumblebees are key pollinators on heathlands (Forup *et al.* Submitted). Natural bumblebee populations support a phylogenetically diverse array of parasites and parasitoids (Alford 1975), some of which can be transmitted vertically within or horizontally between colonies (Schmid-Hempel & Schmid-Hempel 1993; Durrer & Schmid-Hempel 1994).

The natural enemies sampled include five species of mites, the majority of which attach to the exoskeleton of their host, using them to transfer between bees at flowers or when in the colony (Schwarz & Huck 1997). Such phoretic mites are relatively benign, though can be an energetic burden to their host when at high densities. However the tracheal mite *Locustacarus buchneri* (Acarina: Podapolipidae) can cause lethargy and cessation of foraging in their hosts, as they attach and feed directly on their host within their tracheal system (Alford 1975).

Pathogenic parasites include three species of gut protozoa, *Crithidia bombi* (Zoomastigophorea: Trypanosomatidae), *Nosema bombi* (Microsporidia: Nosematidae) and *Apicystis bombi* (Neogregarinida: Ophrocystidae). Impacts to their host include diarrhoea, reduction in foraging efficiency, ovary size and colony growth rate, alteration in timing of hibernation and emergence, and disintegration of the fat body (reviewed by Schmid-Hempel (1998). Intra-colony transmission occurs passively following the ingestion of infective spores that are shed in the faeces of an infected individual. This can be relatively quick in dense aggregations of hosts at the nest (Schmid-Hempel & Schmid-Hempel 1993; Schmid-Hempel & Stauffer 1998). Infected queens that overwinter and found their own colonies subsequently facilitate transmission of protozoa through host generations (Liu *et al.* 1974; Shykoff & Schmid-Hempel 1991b). Inter-colony transmission via the shared use of floral resources contaminated previously by infected foragers has been documented for the relatively benign *C. bombi* (Durrer & Schmid-Hempel 1994). This may also be the

case for *N. bombi,* as healthy bumblebee colonies become quickly infected upon transfer to the field (Imhoof & Schmid-Hempel 1999). With spore viability deteriorating with time from desiccation and UV exposure (Canning 1982), the frequency of host visitation to contaminated floral resources may be a strong determinant for inter-colony transmission.

Bumblebees also harbour both dipteran and hymenopteran parasitoids. The dipteran Conopidae patrol floral patches from June to August for foraging *Bombus* hosts. They attack and oviposit into the abdomen of their host whilst in mid-flight or when foraging. The parasitoid wasp *Syntretus splendidus* (Hymenoptera: Braconidae) similarly oviposits into a bumblebee host, though such activity occurs much earlier in the season relative to the Conopidae (Alford 1975). The larvae of both parasitoid species develop and consume their host from within prior to emergence (Alford 1975; Clements 1997).

Site selection & protocol

Data collection was conducted between June and September 2005 on twelve heathland fragments in south Dorset, England (Table 3.1). Using a matched pairs design, six 'ancient' heathland sites were used as reference sites and paired to six 'restored' sites (Refer to Chapter 2 for their definitions). Afforested heathland in south Dorset, such as the restored sites prior to their restoration, typically support minimal floral resources as a result of dense shading, and bumblebee visitation was seen to be scarce at best (Henson & Memmott, pers. obs.). Previous work by Moore (1962) found the survival of heathland indicator species (including insects, reptiles and birds) to be poor in afforested heaths, with species limited only to plantation rides and edges and reduced relative to shading density. Consequently I did not include afforested heathland as a second site treatment effect after restoration, and assume the ecological restoration program itself to be responsible for the vast majority of any recolonisation by the heathland community thereafter. Ancient (control) and restored (treatment) sites were paired according to geographic location, management intensity and management technique (e.g. grazing and burning). Pine forest, dense gorse hedges, and / or road boundaries separated sites within each pair. Central within each heathland site, plots 100m x 100m in size were selected.

Plots within a pair were separated by distances ranging between 0.5 to 3.6 kilometres, with pairs separated by 2 to 20 kilometres. The variation in separation distances were as a consequence of the continual fragmentation of remnant heathland by urban expansion. Where possible, these distances were maximized to reduce the likelihood of shared bumblebee colonies. Central within each site sampling plots 100m x 100m in size were selected, and ten 100m long transects spaced 10m along the baseline of each plot were divided into two blocks of five transects each, for sampling floral and bumblebee abundance as described below. Fieldwork was conducted approximately every eleven to sixteen days from June to September 2005, with a total of five sampling sessions quantifying flower, bumblebee, and bumblebee parasite, pathogen, and parasitoid abundance.

Floral sampling

Two 100m long transects (one from each block) were surveyed at each plot per sampling session for flowers using a 0.5m x 0.5m quadrat placed at 0, 15, 30, 45, 60 and 75 metres along each transect. At each point, the average number of flowers per m^2 for all species present was recorded. For the dominant plant species *Erica tetralix*, *Erica cinerea,* and *Calluna vulgaris*, floral abundance was estimated by multiplying the mean number of flowers per floral spike by the number of floral spikes present per m^2.

***Bombus* sampling**

Foraging bumblebees were sampled from both transects used in the floral surveys as well as from a third transect selected at random per sampling session. Both sites of a pair were sampled on the same day, and sampling only took place under calm, warm and dry conditions. Sampling was alternated through morning (08:30-11:30), early afternoon (11:30-14:30) or late afternoon (14:30-17:30) throughout the season. Each transect was walked twice and all bumblebees foraging from flowers within 1 metre either side of the transect line were collected. The flower species and transect the individual was found foraging upon was recorded. Queen bumblebees were avoided to reduce impact upon colony densities. Bumblebees were caught using a sweep net

or captured directly into a glass vial lined with a paper bag to minimise loss of external parasites and pollen. Vials were kept in a cool box and transferred to a commercial freezer at the end of the sampling day to kill the bumblebees and ensure preservation of their natural enemies. Samples were stored at –20 degrees Celsius prior to being dissected at the end of the sampling season.

Parasite, pathogen and parasitoid sampling

Individual bumblebees were identified to species before being surveyed for natural enemies. Mites attached to the external surface of the hosts were collected and identified using Alford (1975). Both forewings of each individual was mounted onto glass slides, and the length from the wing base (proximal end of the median plate) to the distal margin of the radial cell was used as a proximate measure of host size (Harder 1982). The abdomen of the bumblebee was separated from the thorax using forceps and placed in an excavated glass block containing water. The sternites of the abdomen were then gently separated under the dissecting microscope exposing the abdominal cavity. This cavity was thoroughly searched for eggs, larvae, and puparia of the Dipteran parasitoid in the family Conopidae. Identifying conopid larvae to species remains problematic, as limited keys are available. Moreover larvae cannot be reared out to adults for identification, as this would prevent the identification of other natural enemies. The abdominal cavity was also searched for the larvae of the parasitoid wasp, *Syntretus splendidus*. The abundance, location found in their host, and development stage of these parasitoids were recorded.

Air sacs and tracheae were searched for the presence of the tracheal mite *Locustacarus buchneri*, and their abundance and development stage recorded. A section of the mid-gut, hind-gut, Malpighian tubules and fat body were examined for the presence and location of the three protozoan parasites, *C. bombi*, *N. bombi*, and *A. bombi* using a compound light microscope at x200 and x400 magnification. Particular attention was paid to searching the Malpighian tubules for *N. bombi* and the fat bodies for *A. bombi*. The identification of all parasite species was aided by use of reference collections, photographs, demonstrations of live material by Dr Mark Brown (Trinity College, Dublin) and Dr Christine Müller (University of Zurich) and

by use of the literature (e.g. Wallace 1966; Smith 1969; Liu *et al.* 1974; Alford 1975; Lipa & Triggiani 1988; Macfarlane *et al.* 1995; McIvor & Malone 1995).

Data analyses

Minitab version 14.13 (Minitab Inc. 2004) was used for the analyses. Paired t-tests and Wilcoxon signed rank tests were used to assess the effect of restoration treatment on floral, bumblebee and natural enemy parameters. All proportional data were arcsine-root transformed prior to analysis. Regression and correlation analyses were used to relate bumblebee and conopid larvae abundance to resource availability.

Quantitative interaction webs were drawn using a program written in Mathematica^{TM}. Food web statistics calculated for the tri-trophic network comprise: the mean number of bumblebee species per plant species and the mean number of parasite/parasitoid species per host, the number of realised links, l_r (the number of trophic interactions between species in the network), linkage density, L (calculated as $l_r/(p+h+pl)$, where 'p' is the number of parasite species, 'h' the number of host species, and 'pl' the number of plant species present in the network), and connectance, C. Connectance is the number of realised links (l_r) relative to the total number of links potentially possible (l_{max}), i.e $C = l_r/l_{max}$; its value bounded between 0 and 1. In food webs, l_{max} is usually calculated as S^2 where S is the total number of species present in the network. This 'directed' connectance, Cd, incorporates feeding within species at a given trophic level (e.g. pollinators feeding on pollinators) that is not experienced in the network described here. More appropriately, in realistic connectance, Cr (the measure I use here), alternative measures of l_{max} have been calculated, such as h*p in host-parasitoid webs (where 'h' and 'p' are the number of host and parasitoid species present respectively *(*Lewis et al. *2002)*, or i*p in insect-plant webs (where 'i' and 'p' are the number of insect and plant species present respectively (Jordano 1987). Cr was modified to incorporate three trophic levels in this study, such that $l_{max}=(h*p)+(h*pl)$. Modifying connectance to exclude illogical links is crucial in understanding this key metric (Lafferty et al. 2006), as connectance affects food web stability and community resilience to species loss (Dunne et al. 2002). Furthermore connectance is common currency when comparing food webs. Connectance data were arcsine-root transformed prior to analysis.

3.3 Results

Q1) Is restoration of the lower trophic levels more complete than the upper trophic levels of the community?

Restoration of the plant trophic level

Species composition for all sites included the key ericaceous heathland flowering plants *Erica cinerea, Erica tetralix* and *Calluna vulgaris*, and the gorse species *Ulex europaeus, Ulex gallii* and *Ulex minor* (Fabaceae). Total floral abundance for the season was not different between ancient and restored heathland sites of a pair (Paired t test, $t = 0.06$, $p = 0.953$), offering on average 2606 ± 215.43 (mean ± SE) flowers/m^2 in ancient and 2579.03 ± 307.99 flowers/m^2 in restored sites, with peak bloom occurring in mid August. Differences can be seen however when considering individual species and their phenology (Figure 3.1). *E. cinerea* flowered earlier in the season and was significantly more abundant in ancient than restored sites of the pair (Paired t-test, $t = 4.96$; $p = 0.004$), whilst the opposite was true for *C. vulgaris,* flowering later in the season, and three times more abundantly in the restored than ancient site of a pair (Paired t-test, $t = -2.84$, $p = 0.036$). All three *Ulex* species occurred at low abundance and when combined for analysis did not differ in abundance between ancient (88.45 ± 32.47 flowers/m^2) and restored (72.09 ± 29.29 flowers/m^2) sites of a pair (Paired t-test, $t = 0.57$, $p = 0.590$).

Restoration of the pollinator trophic level

A total of 1756 bumblebees were sampled over the season from all sites, comprising nine species. These included the BAP species *Bombus humilis* and the heath specialist, *B. jonellus*. *Bombus lucorum* and *B. terrestris* together comprised 67.08% ± 11.68% of total host abundance in ancient and 71.17% ± 13.66% in restored sites, followed by *B. lapidarius* (13.34% ± 3.44 & 6.93% ± 2.41 respectively), *B. pascuorum* (8.61% ± 1.12 & 8.68% ± 2.12 respectively), *B. jonellus* (5.43% ± 2.03 & 10.23% ± 4.92 respectively), *B. pratorum* (2.08% ± 1.98 & 0.89% ± 0.39

respectively), & *B. humilis* (1.79% ± 0.63 & 3.14% ± 1.29 respectively). *B. hortorum* and the cuckoo bee, *B. vestalis,* made up less than 1% of foragers. Neither species richness or mean abundance differed between ancient and restored sites of a pair for the season (Table 3.2), with 54% of all foragers sampled from ancient heaths and 46% sampled from restored heaths. The proportional abundance of each species sampled also did not differ significantly between ancient and restored sites (data not shown). Bumblebee abundance was not directly related to mean floral resources (Regression analysis (data from transects 1 & 2 only): $r^2 = 0\%$, $F_{1,10} = 0$, $p = 0.981$; regression equation: *Bombus* abundance = 95.6 + 0.0004 mean floral abundance). Also, no differences were found in host quality between ancient and restored sites per caste per species, when using host size as a proximate measure of host quality (Wilcoxon signed rank test, males: W = 101.0, p = 0.417; workers: W = 178.0, p = 0.432).

Restoration of the parasite, parasitoid and pathogen trophic level

Overall there was no significant difference in levels of parasitism between ancient and restored sites. 49.02% ± 1.71 of bumblebee foragers in ancient, and 42.24% ± 7.54 in restored sites carried one or more parasites either internally (parasites contained within the exoskeleton of the host including tracheal mites), or externally (attached on the exoskeleton) (Table 3.2).

The mite species *Parasitellus fucorum (*Mesostigmata: Parasitidae), *Hypoaspis spp. (*Mesostigmata: Hypoaspidae), *Scutacarus spp. (*Prostigmata: Scutacaridae) and *Kuzinia spp.* (Astigmata: Acaridae), were all found attached to the exoskeleton of *Bombus* foragers. There were no significant differences between ancient and restored sites in mean total mite abundance, mite species richness, prevalence of mite parasitism, or average mite load per individual (Table 3.2). Externally parasitized hosts carried an average of 10.59 ± 2.75 mites in ancient sites and 8.6 ± 1.65 mites in restored sites.

Overall, six internal species including the Conopid fly and Braconid wasp parasitoids, the tracheal mite *L. buchneri,* and the gut protozoans *C. bombi, N. bombi*, and *A. bombi,* were recorded in *Bombus* hosts sampled from ancient and restored heathlands. While mean internal parasite species richness did not differ

significantly between the site types of the pairs, parasite prevalence was significantly greater for hosts from ancient than restored heaths of the pair (Table 3.2). This difference was largely attributed to parasitism by conopid larvae (Figure 3.2), as removal of this group from the analysis resulted in the loss of this significance (Table 3.2). Parasitism by more than one internal species was found in 3.18% ± 0.73 and 1.64% ± 0.58 of hosts in ancient and restored sites respectively (Table 3.2).

Conopidae could not be identified to species from the larvae, although adults of the conopid *Sicus ferrugineus* were observed patrolling both *E. cinerea* and *C. vulgaris* flowers during the field season as well as in previous years (Chapter 2). Conopid host range, that is the number of *Bombus* species parasitized, was significantly larger in ancient than restored heaths of a pair (mean of 4.83 ± 0.40 hosts in ancient sites and 3 ± 0.58 hosts in restored; Wilcoxon signed rank test, W = 21, p = 0.036). Ancient heaths also harboured greater mean abundances of conopid larvae than in restored heaths, whilst their hosts were subject to greater prevalences of attack, carried higher mean conopid loads, and suffered greater levels of superparasitism, i.e. parasitism by more than one conopid individual (Table 3.2). Total conopid larvae abundance was not related to floral abundance (Pearson product-moment correlation (data from transects 1 & 2 only): r = 0.148, d.f. = 10, p = 0.6460), despite adult conopids relying on pollen and nectar for forage (Smith 1969). However, 51.6% of variation in conopid larvae abundance could be attributed to host availability (Regression analysis, $r^2 = 51.6\%$, $F_{1,10} = 10.65$, p = 0.009; regression equation conopid larvae abundance = -18.10 + 0.434 * *Bombus* abundance).

Only three bumblebees harboured larvae of the braconid wasp *S. splendidus*, and were sampled in the very first session in June. Prevalence of the tracheal mite *L. buchneri*, and the protozoan species *C. bombi, N. bombi,* and *A. bombi* were generally low, and did not differ between ancient and restored heathlands (Table 3.2). The proportion of hosts harbouring one or more protozoan species also did not differ significantly between site types of the pairs (Table 3.2).

Q2) How are the measures of network topology affected?

The communities of the heathland flora, bumblebee pollinators and their parasites were linked together by a complex network of interactions (Figure 3.3, Appendix B). No differences were found in the mean number of bumblebee species per plant species in ancient and restored sites (Table 3.3). However, the mean number of parasites per bumblebee host was significantly greater in the ancient sites of the pairs (Table 3.3). Consequently, the total number of realised links between all three trophic levels, (l_r), was significantly greater in ancient than restored sites of the pairs (Table 3.3), despite no significant differences in the maximum number of unique links possible (l_{max}) between site types of the pairs (Table 3.3). However neither linkage density (L) nor measures of connectance (Cr or Cd) differed significantly between ancient and restored sites of the pairs (Table 3.3).

3.4 Discussion

The floral community, host bumblebee community, and the majority of the natural enemy community were all found to be successfully reinstated at all six restoration heathland projects relative to their baseline reference communities sampled at ancient heathland sites. However the reestablishment of one of the parasitoids, the dipteran Conopidae, remained incomplete. These differences were substantial enough to impact upon web topology measures, leading to significant differences in the realized linkage of the tri-trophic interaction networks of the communities studied. Below I discuss the main findings of this study according to the two main hypotheses set out in the introduction of this chapter, investigating the restoration of the trophic levels themselves, and the impact of this restoration on network topology of the communities.

Q1) Is restoration of the lower trophic levels more complete than the upper trophic levels of the community?

Ancient and restored heathland sites did not differ in their diversity and total abundance of floral resources, suggesting successful re-establishment of floral structure to the community. However differences in relative abundance and phenology of the dominating *Erica* spp. and *Calluna vulgaris* led to a scarcity of flowers early on in the season at restored relative to ancient sites, particularly till the end of July. Such differences in resource availability over the season may potentially affect visitation interactions from bumblebee foragers, as well as their natural enemies. In particular, phoretic mites and the gut protozoan *C. bombi* (and potentially *N. bombi*) are able to transfer between hosts at flowers, and adult parasitoid conopid flies rely on floral resources for forage whilst also patrolling flowers for visiting bumblebee hosts. Yet bumblebee abundance, species richness, and host quality did not to differ between site types, suggesting that bumblebees were not affected by this lag in flowering at restored heaths. The prevalence of overall bumblebee parasitism was also similar between restored and ancient sites, with up to half of *Bombus* foragers found parasitized by a maximum of four external

and six internal parasite species. Levels of parasitism by external phoretic mites and all three gut protozoan species were not different between restored and ancient heaths, indicating their full establishment in the restored community.

However conopid larval abundance, prevalence, load and frequency of superparasitism were all much reduced at restored relative to ancient sites, suggesting the incomplete reinstatement of parasitoid-host interactions despite more than fifteen years of restorative management. Furthermore, the host range of the conopid flies were also consistently narrower at restored sites, despite the similarity in abundance and diversity of hosts available at both restored and ancient heath sites, and evidence from other field data showing their distribution amongst host species to be relative to the proportional abundance of their hosts at a given site (Schmid-Hempel *et al.* 1990a). However the lag in floral resources at restored sites compared to ancient sites may have affected conopid establishment, with conopid food resources, that is their bumblebee hosts (larval food source) and flowers (adult food source), expected to be major determinants in long-term conopid success. The emergence of adult conopid flies in spring and early summer only just coincides with the beginning of flowering at ancient heathlands in mid June, whilst at restored sites, the availability of floral resources is further delayed until mid summer. Thus the establishment of adult Conopidae at heathlands may have been weaker at restored sites due to the delayed availability of floral resources themselves. However although adult abundance was not assessed here, larval abundance did not relate to floral abundance over the season, whilst host availability only accounted for just over half of the variation in larvae abundance.

Other subtle ecological factors therefore must also contribute to the reduced establishment of conopid populations relative to other natural enemies of bumblebees. While conopid flies are highly mobile and effective at tracking host resources, they are limited to accessing foraging hosts at or near their floral resource. External phoretic mites and gut protozoans may appear to be less mobile as they cannot fly, but their high reproductive rate and ability for intra-colony transmission may facilitate their establishment in a restoration programme. Intra-colony transmission of gut protozoans as a result of contaminated nest material can be relatively quick in dense aggregations of hosts at the nest (Schmid-Hempel *et al.* 1990a; Schmid-Hempel & Schmid-Hempel 1993), whilst infected queens that overwinter and infect their own colonies subsequently facilitate transmission of

protozoans through host generations (Liu *et al.* 1974; Shykoff & Schmid-Hempel 1991b). *C. bombi* also has the potential for inter-colony transfer via the shared use of flowers, although spore viability has been found to deteriorate with time from desiccation and UV exposure (Canning 1982; Schmid-Hempel *et al.* 1999). Thus the frequency of host visitation to contaminated floral resources is therefore a strong determinant for their inter-colony transmission. The potential for both mite and protozoan populations to rapidly build up within a nest may therefore buffer any environmental stochasticity and facilitate their establishment and persistence following habitat restoration.

My interpretation of host-parasite interactions is limited by the fact that the number of bumblebee colonies represented by the samples remains unknown. Thus it is possible that although bumblebee abundance did not differ between site types, the number of colonies sampled may have differed, affecting the analyses of host parasite loads, particularly for those natural enemies capable of intra-colony transmission. However research indicates that bumblebee populations foraging on a single patch are likely to represent a relatively high number of colonies (Chapman *et al.* 2003; Darvill *et al.* 2004), and that the likelihood of sampling sister pairs within or at adjacent sites is low. Knight *et al.* (2005) found a maximum of 5% of all pairs within a site to be sisters, whilst only 0.4% of pairs were found to be sisters at sites 500m apart). Also unknown is the extent to which colonies were shared between heaths, i.e. workers of the same colony foraging from both site types of a pair. Dense pine forest or hedges separate sites, but Kreyer *et al.* (2004) suggest such barriers to be ineffective for foraging bumblebees. An effort was made to maximise distances between plots of the pairs to reduce this likelihood of colony sharing. However heathland restoration projects typically select areas adjacent to ancient heaths, such that plots within some pairs were separated by only 500m. Optimal foraging theory and past empirical studies suggest that bumblebees forage close to the nest (Heinrich 1979; Bowers 1985). However this generality has been questioned (Dramstad 1996), and indeed recent use of molecular techniques have determined estimates of maximum foraging distances of *Bombus* species from their nest as follows: 758m for *B. terrestris*, 674m for *B. pratorum*, 450m for *B. lapidarius*, and 449m for *B. pascuorum* (Knight *et al.* 2005). Colony sharing for the three pairs separated by 500m (Table 3.1) is therefore conceivable.

Q2) How are the measures of network topology affected?

The use of quantitative interaction networks enabled a more holistic approach for assessing natural communities when compared to focussing on the presence or absence of component species alone. By studying a substantial section of these communities in the context of habitat restoration, these networks provided the opportunity to identify discrepancies between species interactions of ancient and restored communities in the field. I found that ancient sites supported a significantly higher number of parasite species per host species, and therefore a significantly greater number of unique interactions. These measures reflect incomplete establishment of the upper trophic levels at restored sites relative to ancient, as predicted. The effect on web topology depended upon which statistics were being used. Thus whilst both the mean number of parasite species per host species and realized linkage significantly differed between ancient and restored sites, measures of connectance did not. A previous study by Forup *et al.* (in press) did find differences in connectance between four of the six pairs of heathlands used in this study when investigating plant-pollinator interactions. Although not going beyond the second trophic level, their networks were more speciose, considering visiting Diptera, Coleoptera, Lepidoptera, and solitary Hymenoptera in addition to *Bombus* species. Their differences in connectance however did not cause a decrease in network robustness to perturbation (i.e. the removal of species), as predicted by recent theory (Dunne *et al.* 2002).

The data from this study suggest that plant-pollinator and the majority of host-parasite interactions have been successfully reinstated following over fifteen years of ecological restoration management. Although levels of conopid parasitism and realised linkage in the networks of restored sites were reduced relative to ancient heathland communities, measures of community structure (component species) and the majority of measures of function (floral visitation and levels of parasitism by most natural enemies) were not different. I can be sure that restoration was responsible for the return of the heathland vegetation structure, given the obvious structural differences between the restored heathland and the former pine forest. However it is possible, though very unlikely, that both the bumblebee community and their natural enemies could have persisted through both the afforestation and

restoration process, such that they are communities degraded through disturbance, rather than communities reinstated through restoration. However bumblebees foraging on the minimal floral resources present in patches of afforested heathland was rarely observed, and studies indicate preferences of nest-searching queens to be for open landscapes (Diekotter *et al.* 2006), or along field boundaries that offer tussock-type vegetation or banks (Kells & Goulson 2003). The likelihood of re-establishing colonies annually in or within foraging distance of afforested heaths therefore seems low. Furthermore the reestablishment of their natural enemies will be lower due to their reduced dispersal ability and tolerance to environmental perturbations.

Considering parasites, pathogens and parasitoids in evaluating restoration success

Parasites are frequently ignored in community ecology (Huxham *et al.*1995; Marcogliese & Cone 1997; Dobson *et al.* 2006), despite their role in structuring ecological communities (Dobson & Hudson 1986; Minchella & Scott 1991), influencing biodiversity and community functioning (Mouritsen & Poulin 2002; Montoya *et al.* 2003; Summers *et al.* 2003; Prenter *et al.* 2004; Mouillot *et al.* 2005). The occurrence of parasites within an interaction network can also provide us with information on their hosts such as their abundance, demography, and trophic position, and are indicative of persistent ecological and seasonal patterns that drive and maintain their parasite-host relationship (Marcogliese & Cone 1997; Marcogliese 2003). Rapid generation times in relation to their host also enable them to adapt and respond quickly to perturbations (see reviews by Lafferty (1997) & Marcogliese (2005)).

Many studies, though mainly of aquatic systems, suggest that parasites are good potential indicators of environmental degradation or stress, but only three investigate their potential for recovery after some degrading effect. Cone, Marcogliese & Watt (1993) found greater species richness and higher numbers of parasitic infections of eels in a limed portion of a watershed following up to six years of artificial buffering against acidity, compared to acidic and mixed portions.

Valtonen *et al.* (2003) found only a few species of fish parasites showing evidence of recovery, comparing communities in lakes sampled before and nine years after chemical loading from a pulp mill was reduced. To my knowledge, only Huspeni & Lafferty's study (2004) specifically uses parasite communities to measure restoration success, comparing trematode communities of snails from control sites to sites sampled before and up to six years after ecological restoration of a salt marsh. My study extends their approach by incorporating quantitative interaction networks in the evaluation of restoration success of a phylogenetically diverse array of parasites in terrestrial communities.

Conclusions

Reinstating the interactions between species is as important as reinstating the species themselves if the aim is to promote an ecologically functioning and sustainable community. The higher trophic levels of a community network have the potential to act as robust indicators of the degree of restoration success, as their reliance upon the lower trophic levels leave them to be the last to establish as a community is restored. Food webs and other ecological networks have not been widely applied to the field of conservation biology, but given the practical and theoretical advances being made in food web construction, along with an ongoing need for habitat restoration, the use ecological networks as a conservation tool could be very timely.

3.5 Tables & Figures

Table 3.1 The 12 heathland sites located in the Poole Basin, South Dorset, used in this study. These six pairs of ancient, reference and restored sites are listed below, together with their grid reference, area of heathland, distance between pairs, elevation (metres above sea level), and their management authority. All sites are fragments of the overall remaining heathland matrix in Dorset (8170 ha.) (jncc.gov.uk).

Site type	Pair	Study site	Grid reference SY/SU/SZ	Grid reference BNG	Study plot (total heath area) (Ha.)	Distance between pair (km)	Elevation (m. asl.)	Ownership / Management
Ancient	1	Arne, Grip Heath	Y97386	87595	30 (310)	0.5	5	RSPB
reference	2	Lower Hyde Heath	Y88321	91053	18 (18)	0.5	25	Private Landowner
heathland site	3	Gore, Great Ovens	Y92616	90141	25 (88)	3.6	19	Herpetological Cons. Trust (HCT)
	4	Morden bog Heath	Y91143	92235	16 (149)	0.5	15	English Nature
	5	Woolsbarrow Fort, Wareham Forest	Y89198	92550	8 (420)	2.2	53	Forestry Commission
	6	Holt Heath, Summerlug Hill	U06854	4798	5 (406)	2.2	30	English Nature
Restored	1	Arne, Grip Heath	Y97349	87747	8 (310)	0.5	7	RSPB
heathland site	2	Lower Hyde Heath	Y87868	90993	2 (15)	0.5	35	Forestry Commission / HCT
	3	Holton Lee Heath	Y95927	91775	15 (58)	3.6	0	Holton Lee Charity / RSPB
	4	Morden Bog Heath	Y90898	92235	8 (149)	0.5	15	English Nature
	5	Wareham Forest	Y91235	90127	3 (420)	2.2	6	Forestry Commission
	6	Whitesheet Plantation	Z11541	99006	14 (42)	2.2	14	Forestry Commission

Table 3.2 Parasite abundance, diversity, and prevalence variables from host bumblebees foraging in the 6 pairs of ancient reference and restored heathland sites. Mean values per variable were calculated across all 6 sites per site type, and are displayed together with their standard error of the mean. Significant p values (quoted at 95% confidence levels) are indicated by * ($p < 0.05$) and ** ($p < 0.005$).

	Ancient, reference sites (n=6)	Restored sites (n=6)	Paired test	Test statistic	p value (95% confidence)	Significance
Bumblebee Hosts						
Total abundance	951.00	805.00				
Mean abundance per site	158.50 ± 24.49	134.17 ± 12.52	Paired t-test	1.12	0.31	
mean species richness per site	6.33 ± 0.49	7 ± 0.26	Paired t-test	-1.20	0.29	
1. Overall Parasitism						
% parasitised (internally or externally)	49.02 ± 1.71	42.24 ± 7.54	Paired t-test	2.11	0.09	
% internally AND externally parasitised	7.85 ± 0.76	4.41 ± 0.83	Paired t-test	2.35	0.07	
2. External mite Parasitism						
Mean total abundance (excluding pair 5)	407.60 ± 152.73	306.80 ± 80.73	Paired t-test	0.61	0.58	
Mean species richness per site	3.00 ± 0.37	3.33 ± 0.21	Paired t-test	-1.00	0.36	
% parasitised externally	23.50 ± 2.79	25.00 ± 3.95	Paired t-test	-0.28	0.79	
Mean mite load per host (excluding pair 5)	2.56 ± 0.64	2.08 ± 0.45	Paired t-test	0.56	0.60	
Mean mite load per parasitised host (excluding pair 5)	10.59 ± 2.75	8.60 ± 1.65	Paired t-test	0.60	0.58	
3. Internal Parasitism						
Mean species richness per site	4.67 ± 0.49	3.80 ± 0.54	Paired t-test	1.54	0.19	
% parasitised internally	33.5 ± 3.64	21.57 ± 2.57	Paired t-test	6.45	0.00	**
% parasitised excluding Conopidae	9.26 ± 0.96	6.59 ± 1.31	Paired t-test	1.66	0.16	
% parasitied by > 1 internal species	3.18 ± 0.73	1.64 ± 0.58	Paired t-test	1.36	0.23	
a) Conopid parasitism						
% parasitised by Conopidae	27.4 ± 3.86	16.83 ± 2.96	Paired t-test	4.35	0.01	**
Conopidae host range	4.83 ± 0.40	3 ± 0.58	Wilcoxon	21.00	0.04	*
Mean abundance of conopid larvae per site	61.33 ± 12.12	29.33 ± 6.92	Paired t-test	3.98	0.01	*
Mean conopid load per host	0.38 ± 0.06	0.21 ± 0.03	Paired t-test	4.61	0.01	**
Mean conopid load per parasitised host	1.40 ± 0.06	1.27 ± 0.03	Paired t-test	1.77	0.14	
% hosts superparasitised by Conopidae	6.88 ± 1.80	3.10 ± 0.75	Paired t-test	4.07	0.01	*
% parasitised hosts superparasitised by Conopidae	24.36 ± 3.37	18.18 ± 3.04	Paired t-test	1.51	0.19	
b) Tracheal mite parasitism						
Mean abundance of tracheal mites per site	72.7 ± 27.0	208 ± 129	Wilcoxon	6.00	0.79	
% parasitised by tracheal mites	0.84 ± 0.31	1.11 ± 0.63	Paired t-test	0.02	0.98	
Mean tracheal mite load of hosts	0.47 ± 0.15	2.20 ± 1.65	Wilcoxon	6.00	0.79	
Mean tracheal mite load of parasitised hosts	80.10 ± 29.90	256 ± 188	Wilcoxon	6.00	0.86	
c) Protozoan parasitism						
% parasitised by protozoan(s)	8.23 ± 0.79	5.29 ± 1.10	Paired t-test	2.08	0.09	
% parasitised by *Crithidia bombi*	6.35 ± 0.82	4.19 ± 1.03	Paired t-test	1.64	1.64	
% parasitised by *Nosema bombi*	0.68 ± 0.23	0.45 ± 0.34	Paired t-test	0.86	0. 430	
% parasitised by *Apicystis bombi*	1.20 ± 0.33	0.65 ± 0.27	Paired t-test	1.25	0.27	

Table 3.3 Network topology summary statistics from the ancient and restored networks. Mean values per variable were calculated across all 6 sites per site type, and are shown along with their standard error of the mean. Significant p values (quoted at 95% confidence levels) are indicated by * ($p < 0.05$) and ** ($p < 0.005$).

	Ancient, reference sites (n=6)	Restored sites (n=6)	Test statistic	p value (95% confidence)	Sig.
Measures of generalisation					
Mean number of bee species per plant species	1.65 ± 0.17	1.86 ± 0.20	-0.61	0.568	
Mean number of parasite species per bee species	1.23 ± 0.08	0.99 ± 0.06	2.9	0.034	*
Food web statistics					
Realized links (lr)	39.16 ± 3.26	33.67 ± 3.56	2.91	0.034	*
Maximum links (lmax)	73.86 ± 11.45	73.43 ± 6.52	0.04	0.966	
Linkage density	2.13 ± 0.06	1.86 ± 0.15	1.78	0.135	
Connectance Cr	0.52 ± 0.03	0.44 ± 0.03	1.33	0.241	

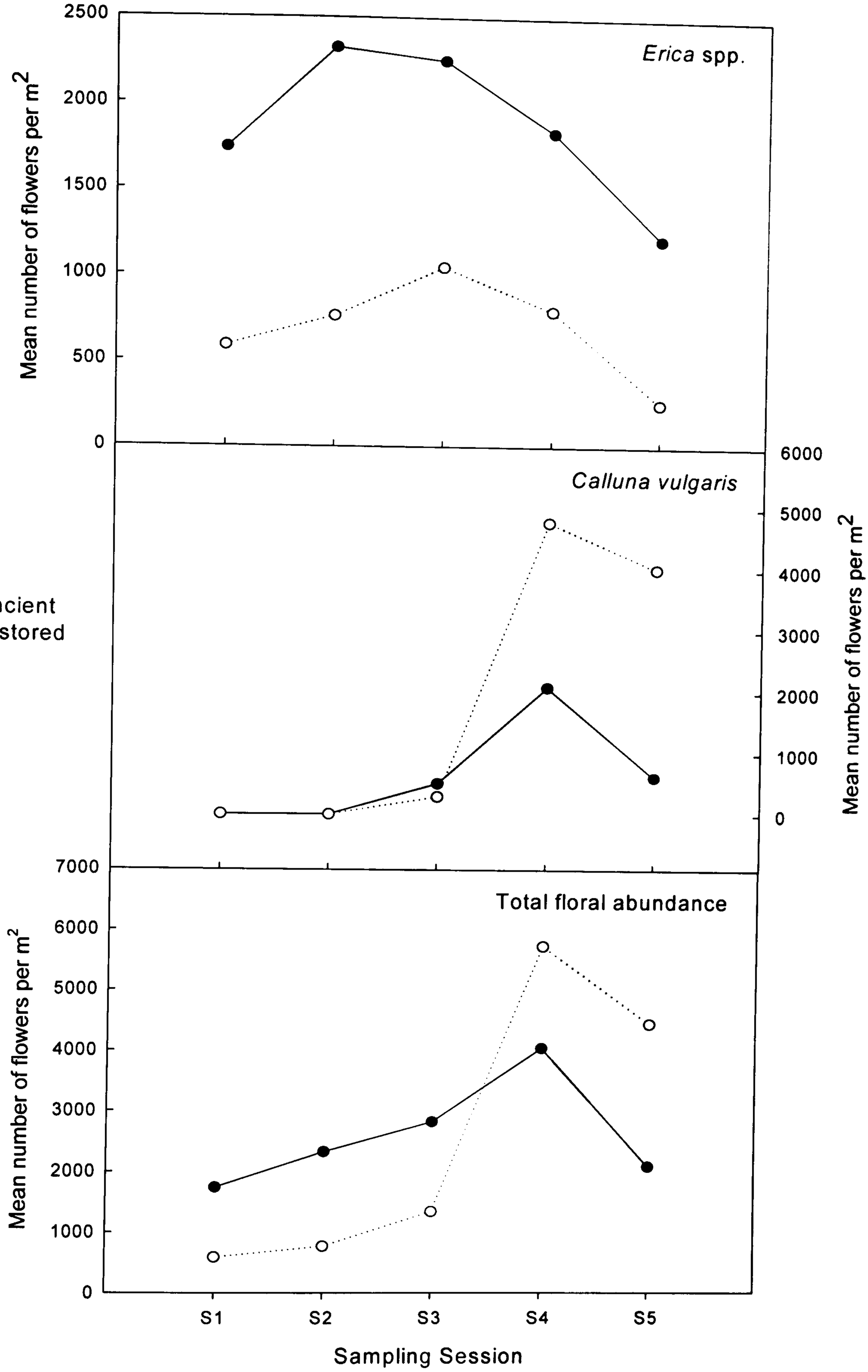


Figure 3.1 The mean floral abundance (per m^2) of *Erica* spp. (*E. cinerea* & *E. tetralix*), *Calluna vulgaris*, and the total floral abundance at ancient and restored sites over the sampling season. Sampling sessions took place in late June (S1), mid July (S2), early August (S3), late August (S4), and mid September (S5).

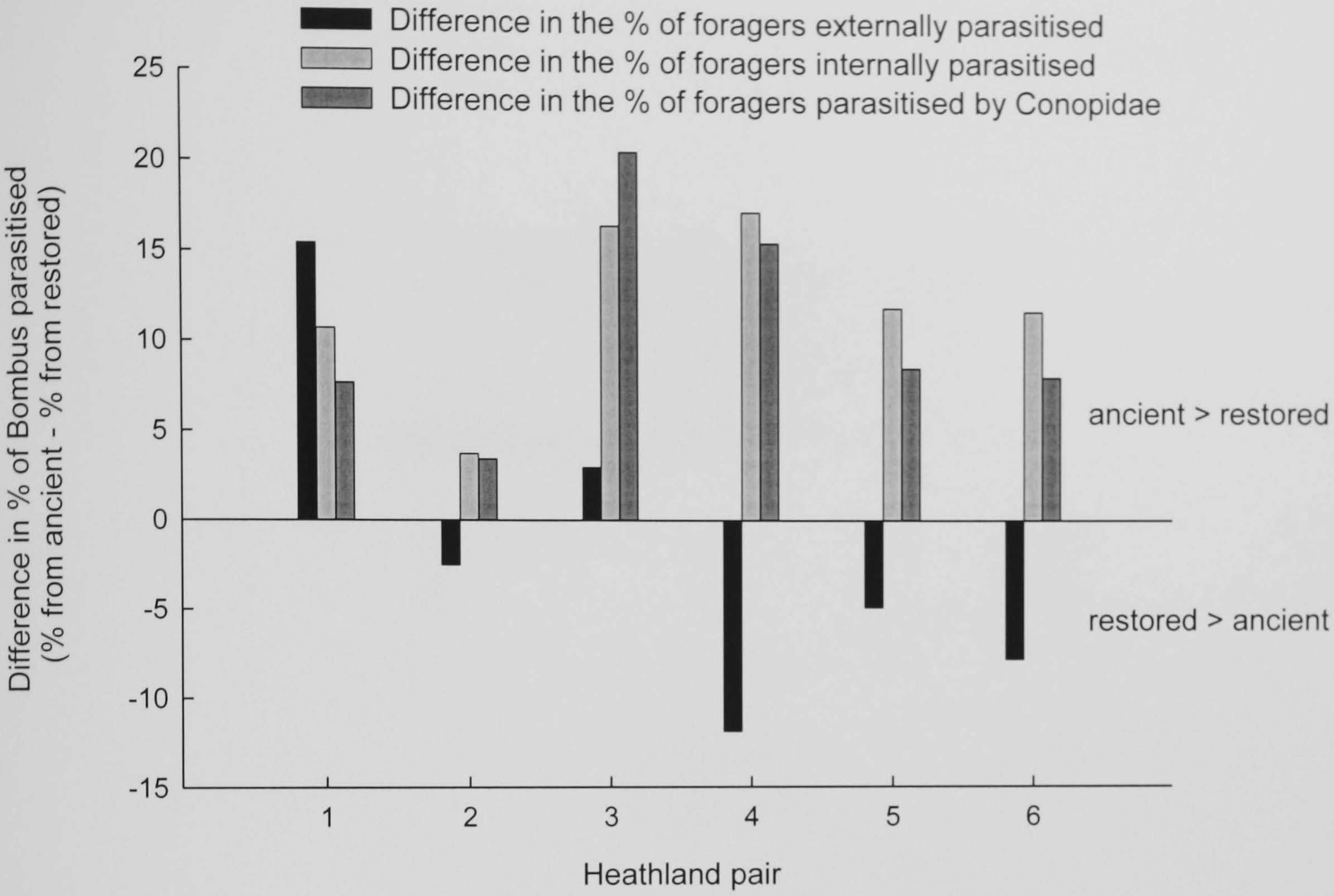


Figure 3.2 The percentage difference in rates of external, internal, and Conopid parasitism of bumblebees, sampled from the 6 pairs of ancient and restored heathland sites. Bars above the line indicate that a greater proportion of foragers in the ancient site of the pair were parasitized than in the restored site; bars below the line indicate the opposite.

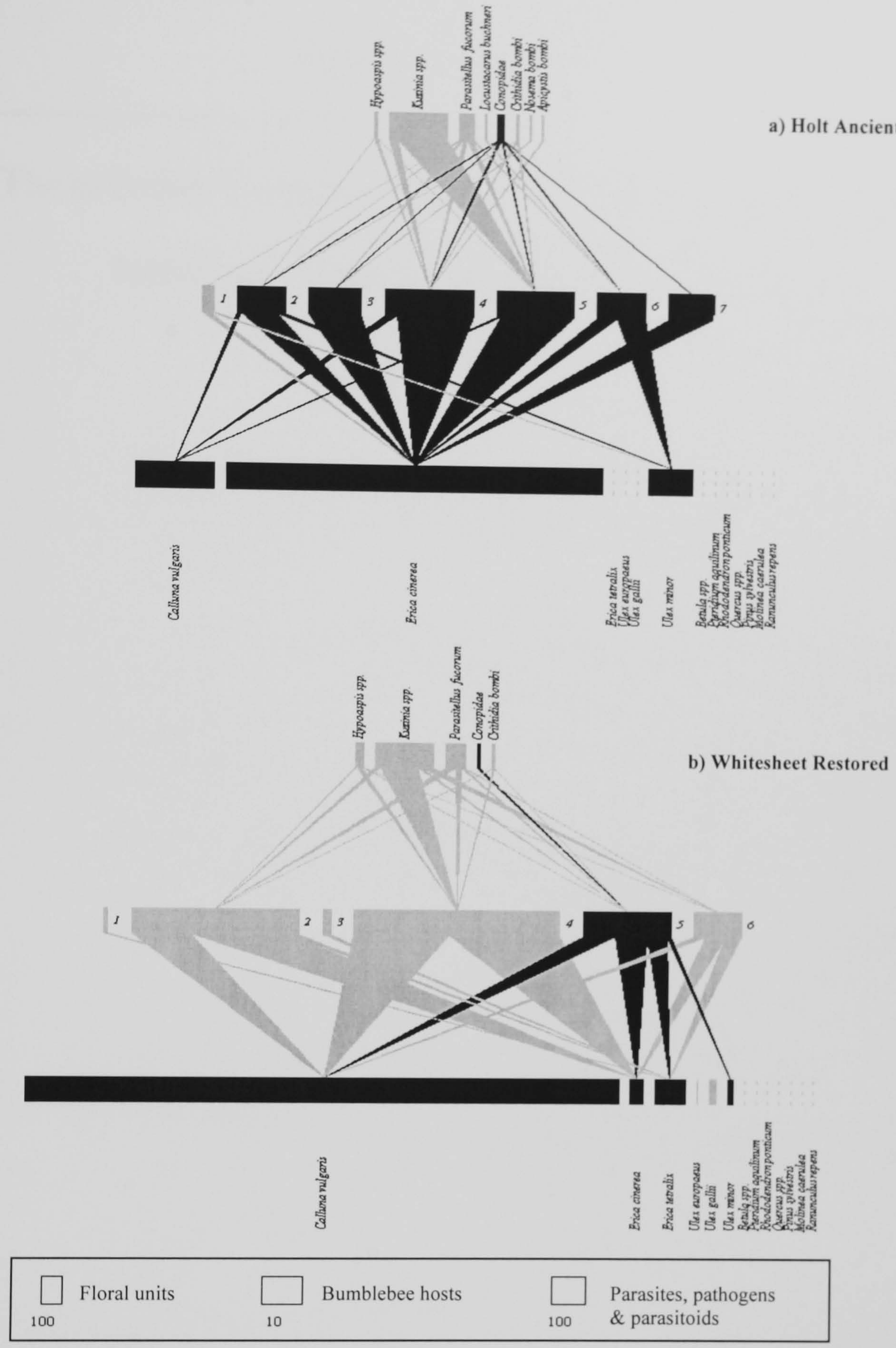


Figure 3.3 Quantitative interaction webs representing three trophic levels for a) Holt ancient and b) Whitesheet restored heathland sites (Pair 6), chosen as being representative of the pairs. The bottom row of rectangular bars represents the flower species; the middle represents the bumblebee species; the top row represents the natural enemies of the bumblebees. Each rectangular bar of the row represents a single species, their relative abundance proportional to bar width. Bumblebee species labelled are: 1) *B. humilis*, 2) *B. jonellus*, 3) *B. lapidarius*, 4) *B. lucorum*, 5) *B. terrestris*, 6) *B. pascuorum* and 7) *B. pratorum*. The scale bar at the base of the figure represent: for flora, 100 floral units per m2 of heathland; for bees, 10 individuals; and for their natural enemies, 100 individuals (with exception of the protozoan parasites *Crithidia, Nosema* and *Apicystis bombi*, where bar width is proportional to the number of hosts infected, as abundance could not be recorded). The solid triangles that connect the trophic levels represent the interactions linking species; the size of the triangle being proportional to the frequency of the interaction. Both webs are drawn to the same scale and the conopid network is highlighted in black. Refer to Appendix B for the remaining five pairs of quantitative interaction webs.

CHAPTER FOUR

The prevalence and patterns of parasitism in bumblebees of heathland communities in south Dorset, U.K.

In: **Henson, K.S.E.** & Memmott, J. Prevalence, patterns and parasitism in bumblebees of heathland communities in south Dorset, U.K. *In prep.*

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CHAPTER FOUR

The prevalence and patterns of parasitism in bumblebees of heathland communities in south Dorset, U.K.

Summary: *While it is well known that bumblebees are economically important pollinators of agriculture, horticulture and floriculture in temperate regions, it is less well appreciated that they are key to the survival of a phylogenetically diverse array of pathogens, parasites and parasitoids. This chapter assesses the prevalence and patterns of attack by these natural enemies, with the aim of updating the existing knowledge and providing baseline data on the extent of parasitism for bumblebees in the UK.*

Twelve heathlands in south Dorset, England, were sampled for bumblebee foragers and their parasite communities. Nine bumblebee host species were found to harbour ten species of natural enemies including ectoparasitic mites, tracheal mites, protozoa, and Dipteran and Hymenopteran parasitoids. A mean of 45.63% of foragers harboured one or more parasite species. Bumblebee workers were significantly more parasitized than males, whilst parasitism by Conopidae was greatest for B. lucorum *and* B. terrestris *and lowest for the heathland specialist,* B. jonellus.

Host size differences between species were a significant confounding factor, with a significant positive relationship between bumblebee worker size and parasitism by Conopidae. With regard to foraging, both parasitized and unparasitized bumblebees appeared to preferentially forage on Erica spp. *than on* Calluna vulgaris. *Parasitism did not correlate with the likelihood of pollen carriage. In this chapter I discuss these results in relation to previous records for bumblebee parasitism, and consider the implications of host decline in the context of their natural enemies.*

4.1 Introduction

Current patterns in the distribution, abundance, and diversity of bumblebees in the United Kingdom are in stark contrast to earlier literature documenting *Bombus subterraneus* as "common" (Sladen 1912), and *B. sylvarum* "everywhere as usual" (Hallett 1928). The former is now one of three species declared extinct, the latter one of four current Biodiversity Action Plan (BAP) priority species for conservation, with a further three species to be considered for BAP inclusion (Edwards & Williams 2004). These seven species are of particular concern, with evidence documenting dramatic reductions in their distributional range by up to 50% when comparing pre- and post- 1960 records (Williams 1982), and their decline still ongoing (Carvell 2002; Edwards & Williams 2004). Indeed only six species of bumblebee remain relatively common in the UK, and similar reductions in abundance and geographical range have also been documented for bumblebee populations in Europe and North America (Williams 1982; Buchmann & Nabhan 1996; Goulson 2003).

Significant losses of these species have implications for the communities in which they live. Their sociality, long flight season, and existence within multi-species assemblages make bumblebees exceptionally effective generalist pollinators, with species partitioning resources via variation in proboscis length (Ranta & Lundberg 1980), nesting requirements (Kells & Goulson 2003) and colony phenology (Pekkarinen 1984). Furthermore, their large size relative to other pollinators enhances their ability for buzz pollination (i.e. using resonant vibration to dislodge pollen that is firmly held by the anthers), whilst allowing thermoregulatory control necessary for periods of foraging in less ideal weather conditions (Corbet *et al.* 1993). Thus bumblebees are economically important pollinators in temperate regions (Williams 1995; Kearns *et al.* 1998), with a substantial percentage of agricultural, horticultural, and floricultural crops being dependent on, or enhanced by, bumblebee pollination (Corbet 1987; Corbet *et al.* 1991; Willmer *et al.* 1994). Moreover, wild plant populations and semi-natural ecosystems of conservation priority such as ancient grasslands and heathlands, are reliant upon bumblebees for cross-pollination (Kwak *et al.* 1991; Osborne *et al.* 1991; Steffan-Dewenter & Tscharntke 1999; Forup & Memmott 2005a).

Rather less discussed however is the community of parasites, pathogens, and parasitoids (hereafter referred to collectively as parasites), that are also reliant upon bumblebees (Alford 1975). Such a diversity of natural enemies is particularly favoured by the high density and low genotypic variation of their bumblebee hosts at the nest, the coexistence of multiple, ecologically similar host species over a season, and the assurance of transmission through host generations via hibernating queens (Schmid-Hempel 1998). Thus declines in bumblebee abundance render these natural enemy communities directly susceptible to extinction, as many are supported exclusively by *Bombus* species. These 'hidden' parasite communities are an important component of biodiversity in their own right, whilst also playing key roles in healthy ecosystems by regulating populations and structuring communities, as discussed in Chapter 3. Parasites can also be useful indicators of ecosystem stability (Marcogliese & Cone 1997b), whilst their absence in certain circumstances may even reduce biodiversity and promote the spread of invasive species (Lafferty 2003).

Factors implicated in the decline of bumblebee populations in the UK include the loss of suitable forage following the intensification of agriculture and associated habitat loss (Goulson *et al.* 2005; Carvell *et al.* 2006), and the increased susceptibility to environmental change in the species that live at the edges of their distributional range (Williams 1986, 1988 & 2005). Other factors less well understood but potentially involved in bee declines, include the impact of pesticides (Williams 1986; Thompson 2001), interspecific competition (Forup & Memmott 2005a), and the loss of suitable nesting and hibernation sites (Goulson 2003). The role of parasitism in the well documented declines of bumblebee abundance and diversity remains unknown however (Sihag 1995). Indeed of all their natural enemies only a small proportion are known to directly cause host fatality (e.g. hymenopteran and dipteran parasitoids); the majority exhibiting low, though variable, impacts on colony fitness and colony size (Shykoff & Schmid-Hempel 1991b; Schmid-Hempel 1998). Furthermore, evidence for the preferential selection of a specific bumblebee host species by their natural enemies remains inconclusive, making it difficult to correlate bumblebee decline with parasitism. However the dipteran parasitoid family, Conopidae, are an exception here, though evidence of their preference for specific species has been confounded by bumblebee size. For example, Otterstatter (2004),

Schmid-Hempel & Schmid-Hempel (1996), and Muller *et al.* (1996) all found larger bumblebees to be more prone to attack, supporting evidence of the greater developmental success of conopid larvae within larger bumblebee hosts (Schmid-Hempel & Schmid-Hempel 1996a; Otterstatter 2004). Conopid preference has also been confounded by bumblebee phenology, with the marked seasonal appearance of conopids found to account for differences amongst bumblebee species resulting in lower levels of parasitism among bumblebee species completing their lifecycles early (Schmid-Hempel *et al.* 1990).

While there is little direct evidence, parasites can be implicated in bumblebee decline, albeit by considering the obverse case – that of their release from their parasites. For example in New Zealand the rapid population expansion of imported bumblebees has been attributed to enemy release (Macfarlane & Griffin 1990; Goulson 2003). Moreover it is known that the pathogenicity of parasites can increase dramatically in resource-stressed hosts, with protozoan infection increasing host mortality by 50% in starved bumblebees (Brown *et al.* 2000). Such increases in susceptibility to parasitism when under poor resource conditions have further ramifications for those declining bumblebee host species already under stress. Furthermore, host genetic heterogeneity can be a crucial determinant in parasite virulence (Imhoof & Schmid-Hempel 1998; Schmid-Hempel 1998) and prevalence (Baer & Schmid-Hempel 1999, 2001), leaving rare and declining species increasingly susceptible to disease as a consequence of their reduced genetic diversity (Ellis *et al.* 2006; Goulson *et al.* 2006). Parasitism can also directly impact upon the pollination services of bumblebees by altering their foraging behaviour. Studies have found that parasitized bumblebee hosts have impaired learning ability and proficiency in handing flowers (Gegear *et al.* 2005) and reduced discriminatory ability between rewarding and unrewarding floral resources (Gegear *et al.* 2006). Parasitism has also been shown to alter the constancy of floral visitation and to reduce the foraging rate of the parasitized bumblebee host (Otterstatter *et al.* 2005), whilst there is also evidence to suggest that parasitized bumblebees switch to flowers that require simpler handling (Schmid-Hempel & Schmid-Hempel 1990). Moreover the capabilities of both nectar and pollen collection by bumblebees can be impaired by parasite load. For example, Schmid-Hempel & Schmid-Hempel (1991) found that

the larvae of dipteran parasitoids restricted the space available for the honey crop and reduced the foraging of the bumblebee due to the additional weight of the developing larvae, with bumblebees harbouring 3rd instar larvae less likely to forage for pollen.

The aim of this chapter is to provide essential baseline data on the patterns and prevalence of parasitism of bumblebee communities. In doing so, I provide the first comprehensive, quantitative assessment of bumblebee parasitism for bumblebee communities in England. I focus on the bumblebee communities and their natural enemies of twelve dry, lowland heathland habitats located in south Dorset, England, asking two questions. First, I ask how species richness, abundance, and prevalence of the natural enemy community are related to host caste, species, and size, and secondly, I ask how parasitism affects the foraging behaviour of their host.

4.2 Methods

Study habitat and species

Heathland is a particularly favourable habitats for bumblebees, with its open landscape, low-growing shrubs, and minimal disturbance providing suitable nesting and hibernation sites (Osborne *et al.* 1991). Furthermore the abundance and relatively long flowering season of the Ericaceae and Fabaceae that dominate heathlands provide highly favourable forage for colony growth (Goulson *et al.* 2005). Indeed pre-1960, bumblebee distribution records document Dorset as being the only vice county supporting all of the seventeen species of bumblebee present in the UK at that time. However post-1960 records saw this reduced to just twelve species (Williams 1982).

Nine bumblebee species are considered in this chapter, and their parasites comprise five species of mites (four attached to the exoskeleton and one located in the host's tracheal system), three species of pathogenic gut parasites (the protozoans *Crithidia bombi*, *Nosema bombi* and *Apicystis bombi*), and two parasitoid species, the dipteran family Conopidae and the braconid wasp *Syntretus splendidus*. The parasites' general ecology and impacts on their hosts were described in Chapter 3.

Site selection & sampling protocol

The twelve field sites and the sampling protocols for flowers, bumblebees and their parasites were described in Chapter 3. Further to this methodology, the presence or absence of pollen in the corbiculae of each bumblebee host was recorded.

Data analyses

The data were analysed using Minitab version 14.13 (Minitab Inc. 2004), and proportional data arcsine-root transformed prior to analysis. Small sample sizes due

to low number of representative bumblebee hosts parasitized by specific parasite species meant that 'types' of parasitism were analysed. These 'types' of parasitism included overall parasitism of an individual, including both internal and external parasite species. This 'overall' parasitism was then further divided into individuals that were either parasitized externally or internally, the latter further broken down into parasitism by pathogenic protozoa or parasitoid Conopidae. Also, throughout the analyses the workers of *B. lucorum* and *B. terrestris* were combined as *B. luc/terr* due to the uncertainties in identifying some workers to species based on genitalia.

One-way Analysis of Variance (ANOVA) was used to compare the prevalence of parasitism between host castes. To test for differences in parasite prevalence between host species, Analysis of Covariance (ANCOVA) using Tukey pairwise comparisons was carried out per host caste, with host size as a covariate (host size measured as the length from the wing base to the distal margin of the radial cell). Due to the small sample sizes of some bumblebee species, this analysis could only be carried out for *B. lucorum, B. terrestris* (workers combined as *B. luc/*terr), *B. lapidarius, B. pascuorum* and *B. jonellus* only. To determine the relationship between host size and parasitism levels, regression analyses were carried out per caste, relating mean parasitism levels per site to mean bumblebee size. Within a bumblebee species, paired T-tests were used to compare host size between parasitized and non-parasitized individuals for each of the species.

One-way ANOVA was used to determine the foraging preference of all bumblebees, parasitized bumblebees, and unparasitized bumblebees between flowers of *Erica spp.* and *C. vulgaris.* As the proportional composition of these flower species significantly differed between ancient and restored heathland sites (discussed in Chapter 3), the analyses were carried out separately for ancient and restored heathlands. Bumblebee worker species included in the analyses were *Bombus luc/terr, B. pascuorum, B. lapidarius* and *B. jonellus.* Finally, the degree of association between pollen collection and the parasitized status of bumblebee foragers was assessed using a Chi Square test.

4.3 Results

Prevalence and phenology of bumblebee parasites

Four external and seven internal parasite species were found to parasitize bumblebee foragers visiting dry lowland heathland habitat in south Dorset (Chapter 3). Their distribution over the 12 heathland sites sampled is illustrated in Table 4.1, whilst Table 4.2 shows their distribution amongst the nine species of bumblebee foragers sampled. Two external and two internal parasite species were ubiquitous, occurring in all twelve field sites, whilst the parasitoid wasp and tracheal mite were found in only two of the nine host bumblebee species sampled.

Eggs, larviform males and larviform and gravid females of the tracheal mite were found within the tracheal system of their host, and are illustrated in Figure 4.1. The three protozoan pathogens *Crithidia bombi*, *Nosema bombi*, and *Apicystis bombi* (Figure 4.2), were concentrated in the mid-gut, hind-gut, Malpighian tubules and in the fat body of their hosts, whilst the parasitoid larvae of the dipteran family Conopidae (Figure 4.3) and the braconid wasp *Syntretus splendidus* (Figure 4.4) were found in the abdominal cavity of the host. Furthermore, the accidental capture of a single *B. terrestris* queen found her to be parasitized by a further internal parasite species, the nematode worm *Sphaerularia bombi* (Figure 4.5).

With exception to the parasitoid Conopidae, no significant differences were found between ancient and restored sites in natural enemy prevalence. Conopid parasitism was found to be greater at ancient sites, with a mean of 27.4% ± 3.86% of bumblebees parasitized compared to 16.8% ± 2.96% at restored sites, as documented in Chapter 3. However the aim of this chapter is to provide baseline data for bumblebee parasitism levels for heathlands. As the heathland matrix of southern England is composed of a diversity of heathland patches varying in both age and in their degree of restoration, I pool together the parasitism data for all twelve sites including parasitism by Conopidae, simply to provide an overall descriptor of baseline parasitism rates for the bumblebee community of lowland heathland in the UK. The mean, median, and range of parasitism found for the foraging bumblebees sampled are recorded in Table 4.3.

Overall, a mean of 45.63% ± 1.97% (standard error of the mean) of all sampled bumblebees were parasitized by one or more species of parasite, pathogen or parasitoid. External parasitism by one or more of the four species of phoretic mites affected 24.25% ± 2.32% of foragers, with parasitized bumblebee hosts carrying a median of 9.78 mites per individual, although for one individual bumblebee host a maximum of 981 individuals of *Kuzinia* spp. was recorded at the end of the season. Indeed this parasite was the most prevalent amongst the external phoretic mites, peaking in abundance in early August (Table 4.4).

Bumblebees that were internally parasitized were predominantly host to parasitoid Conopidae, with a mean of 22.15% ± 2.82% of all bumblebees sampled harbouring larvae, compared to a mean of 7.93% ± 0.87% of bumblebees internally parasitized by other parasite species. Superparasitism (i.e. multiple parasitism of a single host) by conopid larvae was found to occur in 21.27% ± 2.36% of conopid-parasitized hosts, with a maximum of 6 larvae recorded in one bumblebee individual. All larval development stages were found in host bumblebees, with 1st instar larvae being the most abundant developmental stage and puparia the least (Table 4.4). No bumblebee hosts parasitized by conopid eggs were sampled after late August, whilst only bumblebee hosts sampled in late July carried puparia. First, second, and third instar larvae peaked sequentially in abundance from late July to late August (Figure 4.6).

Parasitism by the parasitoid wasp *S. splendidus* was rare in comparison to that from conopids, with only three bumblebee hosts sampled in late June carrying larvae. Tracheal mites were similarly low in prevalence, infecting less than 1% of bumblebees sampled. Those bumblebees that were parasitized though harboured a median of 83 tracheal mite individuals, with one host found to be infested with up to 815 mites. Prevalence of infection by pathogenic protozoa was also relatively low, with a median of 7.85% of bumblebee hosts infected and infection predominantly from *Crithidia bombi*. The other two protozoans, *Apicystis bombi* and *Nosema bombi* were both very rare (Table 4.3). Thirty five percent of the total protozoan-infected hosts were collected in the first week of sampling and declined thereafter. The greatest proportion of *C. bombi*-infected bumblebee hosts were collected at this time,

whilst the few bumblebee individuals infected with *A. bombi* and *N. bombi* were collected in early August (Table 4.4).

Parasitism in relation to bumblebee caste

With regard to levels of overall, internal, conopid, and protozoan parasitism, significantly higher proportions of workers were parasitized than were male bumblebees (one-way ANOVA: overall parasitism: $F_{1,10} = 6.92$, $p = 0.025$; internal parasitism: $F_{1,10} = 12.89$, $p = 0.005$; conopid parasitism: $F_{1,10} = 6.35$, $p = 0.030$; protozoan parasitism: $F_{1,10} = 11.97$, $p = 0.006$). The proportion of males and worker hosts externally parasitized by phoretic mites however were not different (one-way ANOVA: $F_{1,10} = 1.94$, $p = 0.194$).

Parasitism in relation to bumblebee species

To determine whether the prevalence of parasitism differed between species of bumblebees, the proportion of individuals parasitized overall, internally, externally, with Conopidae, or by protozoa for five of the most abundant species sampled (*B. lucorum, B. terrestris* (workers were combined as *B. luc/terr*), *B. lapidarius, B. pascuorum* and *B. jonellus*) were analysed for each caste using an ANCOVA, using bumblebee size as the covariate (Table 4.5).

With regard to worker bumblebees, no significant differences were found between bumblebee species in the proportion of individuals parasitized overall, externally, or by gut protozoa after controlling for bumblebee host size (Table 4.5). However, the proportion of workers internally parasitized did significantly differ between bumblebee host species, affecting a significantly lower proportion of *B. jonellus* workers (5.69% ±4.03%) than workers of *B. pascuorum* (27.38% ± 5.13%) (ANCOVA & Tukey post hoc test for pairwise comparisons: $F_{1,19} = 5.18$, $p = 0.009$). Similarly, the proportion of bumblebees parasitized by Conopidae also significantly differed between bumblebee host species, with lower proportions of *B. jonellus* (2.24% ± 1.65%) found to harbour conopid larvae than *B. pascuorum* (19.91% -

2.05%) or *B. luc/terr* (34.61% ± 3.43%). *B. luc/terr* workers were also more parasitized by Conopidae than *B. lapidarius* (11.17% ± 4.61%) (ANCOVA & Tukey post hoc test for pairwise comparisons: $F_{1,19} = 10.72$, $p < 0.001$). (Table 4.5).

With regard to male bumblebees, bumblebee species did not differ in the proportion of individuals suffering from external or protozoan parasitism (Table 4.5). However fewer males of *B. jonellus* (0.67% ± 0.67%) and *B. pascuorum* (4.76% ± 4.76%) suffered from internal parasitism than did males of *B. lucorum* (16.68% ± 4.39%) or *B. terrestris* (31.37% ± 6.74%). *B. lapidarius* were also less parasitized internally than *B. terrestris,* with only 8.56% ± 5.26% of male foragers parasitized (ANCOVA using the Tukey post hoc test for pairwise comparisons: $F_{1,24} = 12.04$, $p < 0.001$). With regard to males parasitized with Conopidae, significantly greater proportions of males of *B. lucorum* (15.45% ± 4.81%) and *B. terrestris* (28.87% ± 6.11%) were parasitized than were *B. jonellus* and *B. lapidarius* (no conopid parasitism), and *B. pascuorum* (2.38% ± 2.38%) (ANCOVA using the Tukey post hoc test for pairwise comparisons: $F_{1,24} = 24.11$, $p < 0.001$). The proportion of males suffering from overall parasitism also differed between species, though significance was marginal (Table 4.5).

Parasitism in relation to host size

Bumblebee size was a significant covariate when determining the effect of bumblebee species on levels of parasitism. To further determine this relationship, regression analyses were carried out per caste to relate mean parasitism levels per site per species, to their respective mean bumblebee size. Again, overall parasitism was divided into external and internal parasitism, with the latter further divided into parasitism by protozoa and by Conopidae. Where parasitism levels were 0%, an average was taken of their respective host sizes and represented as a single value. This was to avoid an overweighting of the slope at its origin, which may have resulted in a false positive relationship. No relationships were found between the mean size of worker bumblebees per site and the proportion of workers that were parasitized overall, or externally (Figure 4.7a). However, a significant positive

relationship between worker size and the proportion of workers that were internally parasitized was found (Regression analysis; $r^2 = 52.4\%$, $F_{1,19} = 20.9$, $p < 0.001$; regression equation: % internally parasitized = -41.2 + 58.6 worker size) (Figure 4.7a). Significant positive relationships remained between worker size and the prevalence of both protozoan and conopid parasitism, although worker size accounted for a greater proportion of the variance in levels of conopid parasitism (Regression analysis; protozoan parasitism: $r^2 = 29.0\%$, $F_{1,16} = 6.54$, $p = 0.021$ (regression equation: % parasitized by protozoa = -49.4 +54.2 worker size); conopid parasitism: $r^2 = 50.9\%$, $F_{1,19} = 19.67$, $p < 0.001$ (regression equation: % parasitized by Conopidae = -42.3 + 55.0 worker size) (Figure 4.8a). With regard to male bumblebees, no relationship between size and the prevalence of parasitism was found (Figure 4.7b & Figure 4.8b).

Previous analyses here showed parasitism to differ between bumblebee species. Indeed the factor 'species' is a major confounding factor when relating size to parasitism, as the species themselves differ significantly in size. I therefore further compared the mean host size of parasitized (divided into internal and external parasitism), and non-parasitized individuals per site using paired T-tests for each species per caste. However due to small sample sizes in some cases, male *B. lapidarius* and *B. pascuorum* could not be analysed, whilst for male *B. jonellus*, size could only be compared between externally parasitized and non-parasitized individuals. I found externally parasitized workers to be significantly larger than non-parasitized workers (Paired t-test: $T = -2.88$, $p = 0.035$; mean length: 0.92cm ± 0.009cm in parasitized vs. 0.90cm ± 0.006cm in non-parasitized workers). However, when analysing males, internally parasitized *B. lucorum* males were significantly smaller than non-parasitized males (Paired t-test: $T = -3.40$, $p = 0.019$; length: 1.05cm ± 0.009cm in parasitized vs. 1.08cm ± 0.004cm in non-parasitized males). None of the other parasitized bumblebees differed significantly in size to non-parasitized bumblebees of the same caste and species (data not shown).

Parasitism in relation to host foraging

At ancient sites, significantly greater proportions of bumblebee foragers were found foraging on *Erica* species (*E. cinerea* and *E. tetralix* combined) than on *Calluna vulgaris*. This was the case for both unparasitized and parasitized bumblebees, and regardless of their parasitized state (Table 4.6). This was likely due to the significantly greater proportional abundances of *Erica spp.* available at ancient sites, as discussed in Chapter 3. However at restored sites, with exception to those foragers parasitized with gut protozoa, no floral preferences were apparent (Table 4.6), with bumblebees foraging equally at both floral species. This was despite the significant dominance in floral resources by *C. vulgaris* as discussed in Chapter 3. Also, significantly greater proportions of bumblebees infected with gut protozoa at restored sites foraged upon *Erica spp.* and not upon *C. vulgaris* (Table 4.6).

With regard to pollen collection, parasitized bumblebee workers were neither more nor less likely to carry pollen than were non-parasitized workers (Chi^2 test: overall parasitism: $\chi^2 = 0.22$, d.f. = 1, p = 1; external parasitism: $\chi^2 = 1.50$, d.f. = 1, p = 0.20; internal parasitism: $\chi^2 = 2.69$, d.f. = 1, p = 0.20; conopid parasitism: $\chi^2 = 1.62$, d.f. = 1, p = 1; protozoan parasitism: $\chi^2 = 1.32$, d.f. = 1, p = 1). This was also the case when comparing the number of workers harbouring first, second, and third instar conopid larvae to non-parasitized workers, and when comparing workers multiply parasitized by two or more conopid larvae to non-parasitized workers (Chi^2 test: 1^{st} instar: $\chi^2 = 0.49$, d.f. = 1, p = 1; 2^{nd} instar: $\chi^2 = 2.87$, d.f. = 1, p = 0.10; 3^{rd} instar: $\chi^2 = 1.95$, d.f. = 1, p = 0.20; two or more larvae: $\chi^2 = 1.42$, d.f. = 1, p = 1).

4.4 Discussion

This study provides the first comprehensive, quantitative assessment of the extent of parasitism in bumblebee communities in England, focusing on the communities foraging on heathland in south Dorset. Worker bumblebees suffered greater parasitism than did male bumblebees, whilst parasitism by Conopidae was greatest for *Bombus lucorum* and *B. terrestris* individuals and lowest for individuals of the heathland specialist species, *B. jonellus*. Furthermore, a significant positive relationship was found between the size of bumblebee workers and their prevalence of parasitism by Conopidae. Both parasitized and unparasitized bumblebees seemed to preferentially forage on *Erica spp.* than on *Calluna vulgaris*. Parasitism did not correlate with the likelihood of pollen carriage. In this section the limitations of the study are considered and the results are put into context by comparing them with published data from other field studies. I end by discussing the importance of monitoring bumblebee natural enemies in addition to monitoring the bumblebees themselves.

Limitations of the study

The incidence of parasitism by the diverse array of parasites, pathogens and parasitoids documented in this study represent only a portion of the true extent of the natural enemy community supported by bumblebees. By considering only host foragers, I cannot assess the abundance of parasites and commensals of their nests that include further species of Diptera and Hymenoptera (including the cuckoo bumblebees from the subgenus *Psithyrus*), as well as species of Coleoptera and Lepidoptera. This study was also limited to a single season, such that I cannot determine the extent of temporal variation in the natural enemy community, although I do know that a similar proportion of the bumblebees were infected by Conopidae in 2004 (Henson, unpublished data). Finally, by sampling bumblebee foragers from the heathland flora rather than directly from the nest, I am unable to determine how representative the parasitism levels are with regard to their colonies. That is, I am assuming that the foragers sampled in this study represent a diversity of colonies (the

colony being their true effective population size), rather than just one or two, for example. Finding colonies or measuring their abundance however is difficult, though recent molecular techniques have successfully been employed to determine colony density inferred through the frequency of sampling sister pairs (Chapman *et al.* 2003; Darvill *et al.* 2004; Knight *et al.* 2005). These studies have shown that *Bombus* populations foraging at a resource patch are generally representative of a relatively high numbers of colonies. Furthermore, the likelihood of sampling sister pairs has been found to be low both within a site (a maximum of 5% of all pairs found to be sisters), as well as at adjacent sites (a maximum of 0.4% of pairs found to be sisters at sites separated by 500m) (Knight *et al.* 2005). Thus it is likely that the parasitism levels documented in this study are a good representation of their prevalence at the colony level.

Prevalence of parasitism in Dorset Heathlands

Bumblebee parasites were common, with just under half of all bumblebee foragers carrying one or more species of parasite. External parasitism by phoretic mites ranged between 10% - 40%, which is at the lower end of the ranges documented in North America (12% -70%) and Europe (17% - 83%) (Schmid-Hempel 1998). The impacts of these phoretic mites are benign however, feeding off the pollen and detritus within the colony before reproducing and transferring to other bumblebee hosts.

The internal natural enemies exert much greater impacts upon their host bumblebees than the phoretic mites. In particular, the larvae of both Dipteran and Hymenopteran parasitoids feed on the internal organs of their host throughout their development (Alford 1975), eventually leading to host death. The prevalence of the dipteran parasitoid Conopidae in Dorset heathlands suggests a mean loss of 22% ± 2.82% of bumblebee foragers per season as a consequence of this parasitism. Their impact on the colony however is less clear as queens were not sampled in this study. However Schmid-Hempel *et al.* (1990) did not find spring queens to be parasitized by conopid larvae, and assumed this was because of their earlier emergence relative to adult Conopidae who appear from June to August (Alford 1975). The impact of

conopid parasitism on colony founding by spring queens may therefore be minimal. Also, although young queens foraging at the end of the summer prior to their hibernation have been found to be infected with conopids, this is typically to a much lesser degree (Schmid-Hempel & Schmid-Hempel 1988; Schmid-Hempel *et al.* 1990b). However with the parasitism of both workers and male bumblebees relatively common, bumblebee colony size may potentially be reduced over the season itself, with smaller colonies found to produce fewer offspring and therefore fewer queens (Schmid-Hempel & Schmid-Hempel 1988). Furthermore the loss of male bumblebees may reduce the mating potential of young queens produced in the late summer.

The frequency of parasitism by Conopidae found in the Dorset heathlands was similar to the documented frequencies of between 20-30%, though peak seasonal prevalence of up to 70% have been recorded (Schmid-Hempel 1998). However parasitism by the hymenopteran parasitoid, *Syntretus splendidus*, were very low, with prevalence recorded between 0-1.2% of foragers, compared to previous rates recorded for England of 11% - 67% between May and July (Alford 1968). The short flight window of *S. splendidus* early in the season between May and June (Alford 1975), may explain their low occurrence in this study, as high levels of floral resources were only available to potential bumblebee hosts from June onwards, and parasitized individuals were sampled only in late June.

Prevalence of infestation by the tracheal mite *L. buchneri* in this study were also low, ranging between 0 - 4% of *Bombus* hosts at the twelve sites, with a mean prevalence of 0.97% ± 0.34%. Furthermore *L. buchneri* was found to infect only the host species *B. pratorum* and *B. pascuorum.* A study in Switzerland also found these two species to be the only infected species out of a community of nine bumblebee species studied (Shykoff & Schmid-Hempel 1991a). Macfarlane *et al.* (1995) suggested that these species may be more prone to parasitism by *L. buchneri* due to their above-ground nesting behaviour increasing the accessibility of their nests to *L. buchneri*. In addition, their early emergence and colony founding in the season coincides with peak tracheal mite prevalence. However tracheal mite prevalence recorded for host communities in Canada did not support this, with the earliest emerging species uninfested, and highest rates of infestation recorded for the

underground nesting species *B. occidentalis* (Otterstatter & Whidden 2004). Levels of infestation recorded for other host communities range between 0.8-7% in northern Europe, 0-0.02% in New Zealand (Macfarlane *et al.* 1995), and between 0-18% in Alberta, Canada (Otterstatter & Whidden 2004).

Overall the rates of infection by protozoa in the Dorset heathlands were much lower than those documented elsewhere in Europe. Frequencies of infection by the pathogen *C. bombi* ranged between 1.27-8.89% of bumblebee hosts, compared to 10-30% commonly recorded (Schmid-Hempel 1998), and a maximal prevalence of up to 80% recorded for foraging workers (Shykoff & Schmidhempel 1991a). For *N. Bombi*, only 0-2.05% of bumblebee hosts were infected in this study compared rates of 9-53% of bumblebee hosts in Switzerland (Shykoff & Schmidhempel 1991a; Imhoof & Schmid-Hempel 1999), whilst for *A. bombi* 0-2.17% of foragers were infected compared to 3-8% recorded in Europe (Macfarlane et al. 1995).

Parasitism in relation to bumblebee caste, species and size

Workers suffered from a greater level of parasitism than did males, whilst bumblebee host species seemed to be differentially susceptible to internal and conopid parasitism only. Such patterns are common to other European studies (Schmid-Hempel & Schmid-Hempel 1988; Schmid-Hempel *et al.* 1990; Shykoff & Schmidhempel 1991a). In this study, *B. lucorum* and *B. terrestris* workers and males showed much higher incidences than did *B. jonellus, B. pascuorum,* and to a lesser extent, *B. lapidarius*. This was similar to the findings of Schmid-Hempel *et al.* (1990) but contrary to those of Shykoff & Schmid-Hempel (1991a), where highest incidences of conopid parasitism occurred in *B. pascuorum*.

However the extent of the choice of bumblebee host species by Conopidae was confounded by other variables such as their phenological overlap with each other and bumblebee host size. For example, it has been suggested that the appearance of the conopid species *Sicus ferrugineus* and *Physocephala rufipes* by June leave late-emerging bumblebee host species more prone to attack (Schmid-Hempel *et al.* 1990). Both these conopid species were seen as adults in the heathland sites (K. Henson pers. obs.; D. Clements, pers. comm.), and are likely to be amongst

the larval conopids sampled. Indeed *B. lucorum,* a late-emerging bumblebee species of heathlands, suffered from higher than expected incidences of conopid parasitism. It may be however, that *B. lucorum* being one of the most frequently sampled species in this study, was also the more frequently available host to their parasites; that is, the parasites themselves effectively 'sample' abundant species more frequently than rarer ones (Vazquez *et al.* 2005b). Indeed Durrer & Schmid-Hempel (1995) found greater levels of parasitism by Conopidae and *C. bombi* as well as higher parasite species diversity, in the more commonly distributed host species compared to rarer species. More generally, patterns in host-parasite interaction networks have been found to partly result from the distribution of abundance amongst the species themselves (Vazquez *et al.* 2005b).

However both *B. pascuorum* and *B. jonellus* are late emerging species (*B. jonellus,* usually an early-emerging species in the south, has late-emerging queens in heathland to coincide with floral resources (Edwards & Williams 2004)). Yet both species experienced the lowest frequencies of internal and conopid parasitism. These species however, were also the smallest of those analysed, the opposite being true for *B. lucorum* and *B. terrestris*. Thus host size may be a more significant confounding factor than simply phenological overlap between bumblebees and conopids. Indeed bumblebee host size was a significant covariate when assessing the prevalence of conopid parasitism between host species, with a significant positive relationship between bumblebee worker size and conopid parasitism found. This is in line with Muller *et al.* (1996) who found bumblebee hosts parasitized by Conopidae to be significantly larger than those individuals not parasitized. Furthermore, Otterstatter (2004) found bumblebee host size to be a better predictor of conopid size than host species, whilst Schmid-Hempel & Schmid-Hempel (1996) documented greater developmental success of conopid larvae in larger bumblebees. Indeed more generally the body size of hosts has been found to positively correlate with the fitness of their parasites in regard to their fecundity and longevity (reviewed in Godfray (1994)). With regard to Conopidae, all four development stages of their larvae (1^{st}, 2^{nd}, and 3^{rd} instar stages followed by the puparium), occur entirely within the abdomen of their bumblebee host, the adult finally breaking out of the exoskeleton of their bumblebee host the following spring. Larger bumblebee hosts

therefore not only offer greater provisioning of internal resources to the larvae, but also provide a greater developmental space for the final puparium case, both factors increasing the developmental success of the emerging conopid fly. Other studies however have not found this relationship (Schmid-Hempel & Schmid-Hempel 1988; Schmid-Hempel & Muller 1991; Schmid-Hempel & Stauffer 1998).

Parasitism in relation to host foraging

Greater proportions of bumblebees foraged on *Erica spp.* where it was proportionally dominant at ancient sites, regardless of whether they were parasitized. This may indicate that bumblebees forage in proportion to the available resources at these heathlands. Yet at restored sites, where *C. vulgaris* dominated the available resources, the proportions of bumblebees foraging on *C. vulgaris* were no different to those foraging on *Erica spp.*, suggesting that bumblebees preferentially forage at *Erica* flowers when abundant. Also, the lack of difference in flower preference between parasitized and unparasitized individuals at ancient sites suggest that parasitism itself may not impact upon floral choice of the infected individual. This is in contrast to studies by Gegear *et al.* (2005, 2006) whereby high levels of infection by the protozoan *C. bombi* in bumblebee hosts impaired their learning and handling of flowers and their ability to differentiate rewarding from non-rewarding flowers. However it is unlikely that *C. bombi* would have much impact on bumblebee foraging in this study, given their very low incidence of infection in bumblebees here. Also, Schmid-Hempel & Schmid-Hempel (1990) found that bumblebee hosts harbouring conopid larvae chose to forage at floral species that required much simpler handling. Although greater proportions of conopid-parasitized bumblebees at ancient sites foraged upon *Erica spp.*, this is more likely due to its dominance at ancient sites rather than differences in the perceived handling difficulty of *Erica spp.* and *C. vulgaris*, as no differences were found at restored sites. This is similar to the findings of Schmid-Hempel & Stauffer (1998), who also found no association between conopid attacks on bumblebees at particular floral species.

What is not clear from this study however is whether the greater proportions of parasitized bumblebee foragers found at *Erica spp.* at ancient sites increased their

susceptibility to parasitism. For example, pathogenic protozoa and phoretic mites use flowers to transfer between bumblebee hosts, the former transmitting between bumblebees through contaminated nectar. Indeed at restored sites significantly greater proportions of protozoan-parasitized bumblebees were also found to forage at *Erica spp*. The enclosed morphology of the bell-shaped, downward-hanging flowers of *Erica spp.* may provide a more protective environment to such parasites, in contrast to the more open, exposed morphology of *C. vulgaris,* where rates of desiccation may be much higher. Flowers of *Erica spp.* may therefore provide an ideal environment for protozoa as they wait for bumblebees to forage upon the contaminated nectar.

Parasitized worker bumblebees were no less likely to have foraged for pollen at their time of capture than were unparasitized workers. However a previous study by Schmid-Hempel & Schmid-Hempel (1988) found that significantly fewer bumblebee hosts parasitized by Conopidae foraged for pollen, whilst a further study by Schmid-Hempel & Schmid-Hempel (1991) found bumblebee hosts harbouring 3rd instar larvae less likely to forage for pollen than expected compared to non-parasitized bumblebees. They suggested that the additional weight of the third instar larvae, filling the entire abdominal cavity of the bumblebee, imposed immediate costs by reducing the foraging capacity of the parasitized bee. I found no evidence for this in this study, with bumblebee hosts carrying first, second, or third instar larvae or a conopid puparium no less likely to carry pollen than non-parasitized workers. Furthermore, bumblebee hosts superparasitized with more than one conopid larvae also showed no association with the likelihood of pollen carriage.

Bumblebee decline and their natural enemies

The reduction in the availability of suitable forage resources, nesting and hibernation sites for bumblebees as a consequence of habitat loss and fragmentation, together with the current and future impacts of climate change, is likely to increase the levels of stress suffered by bumblebees. The extent to which this affects their susceptibility to disease however, remains unknown. Experimental evidence exists for increased virulence and mortality by the gut protozoan *C. bombi* in resource-stressed

bumblebee hosts (Brown *et al.* 2000), suggesting that parasitism may become an increasingly important factor in their future decline. As a consequence of declines in both bumblebees and honeybees, there has been an increase in the import and export of bumblebee colonies for pollinator services (Macfarlane et al. 1995).

Aside from the threat of hybridization between introduced and native host species and subspecies, and their potential to displace natives (Mitsuhata & Ono 1996; Ings *et al.* 2006), the accidental introduction of their natural enemies together with their host has important implications for resident bumblebee communities. Parasites not currently present in the resident native community may be introduced, such as in Japan where the pathogen *N. bombi* found in imported *B. terrestris* colonies successfully infected native bumblebee species (Niwa *et al.* 2004). Novel pathogenic strains have also been introduced together with their imported bumblebee hosts (Goka *et al.* 2006). Furthermore, evidence suggests that commercially-reared bumblebees suffer from greater levels of parasitism than do wild populations, with the transfer of pathogens from commercial to wild communities likely (Colla *et al.* 2006). Research into the prevalence and impacts of bumblebee natural enemies is therefore increasingly important in light of declining native bumblebee species and the growing commercialisation of bumblebee colonies for global exportation.

Conclusions

Bumblebees are crucial for pollination services globally, but are also key to the survival of a phylogenetically diverse array of pathogens, parasites and parasitoids. Yet unlike their well-studied role in pollination, the prevalence and patterns of such hidden natural enemies in the UK remain poorly understood since their first documentation by Sladen in 1912 and Alford in 1975. The work presented here provides the first comprehensive and quantitative assessment of parasitism of bumblebee foragers in dry lowland heathland. Such baseline data are essential to further our understanding of the impact of parasitism on bumblebee communities in light of their declining populations within the UK, whilst highlighting the otherwise hidden and often ignored biodiversity of bumblebee natural enemies.

4.5 Tables & Figures

Table 4.1 The distribution of bumblebee natural enemy species across twelve sites of dry lowland heathland in south Dorset, England, 2005. Sites were sampled for bumblebee communities and their natural enemies at six pairs of ancient (A) and restored (R) heathland sites. The diamond symbol ♦ indicates the presence of a parasite species harboured by foragers sampled from that heathland site.

		Dry lowland heathland sites											
		A1	R1	A2	R2	A3	R3	A4	R4	A5	R5	A6	R6
Bumblebee natural enemies													
External													
Ectoparasitic mites	*Kuzinia spp.*	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦
	Parasitellus fucorum	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦
	Scutacarus spp.			♦	♦	♦							
	Hypoaspis spp.	♦	♦	♦	♦	♦	♦		♦	♦	♦	♦	♦
Internal													
Tracheal mite	*Locustacarus buchneri*	♦	♦	♦	♦	♦	♦	♦	♦			♦	
Pathogenic protozoa	*Crithidia bombi*	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦
	Nosema bombi				♦	♦	♦	♦		♦		♦	
	Apicystis bombi			♦	♦	♦	♦	♦	♦	♦	♦	♦	
Parasitoids	*Conopidae*	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦
	Syntretus splendidus					♦			♦				
Nematode worm	*Sphaerularia bombi*							♦					

Table 4.2 The distribution of bumblebee natural enemy species across nine species of bumblebee foragers sampled from 12 dry, lowland heathland sites in south Dorset, England, 2005. The diamond symbol ♦ indicates the presence of a parasite species found for each species sampled, and per bumblebee caste (workers (w) and males (m) only; with exception to the nematode worm *S. bombi* sampled from a single *B. terrestris* queen (Q)).

Bumblebee species		*B. luc/terr*	*B. lucorum*	*B. terrestris*	*B. lapidarius*		*B. pascuorum*		*B. jonellus*		*B. humilis*		*B. pratorum*		*B. hortorum*		*B. vestalis*	
Bumblebee caste		w	m	m	w	m	w	m	w	m	w	m	w	m	w	m	w	m
Percentage of total foragers		33.8	51.3	21.4	12.6	8.4	9.4	6.4	6.3	8.8	2.3	3.1	1.4	0.2	0.3	0.0	0.1	0.5
Bumblebee natural enemies																		
External																		
Ectoparasitic mites	*Kuzinia spp.*	♦	♦	♦	♦	♦	♦	♦	♦	♦								♦
	Parasitellus fucorum	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦		♦				
	Scutacarus spp.	♦	♦				♦											
	Hypoaspis spp.	♦	♦	♦	♦		♦	♦	♦	♦	♦				♦			
Internal																		
Tracheal mite	*Locustacarus buchneri*						♦	♦					♦					
Pathogenic protozoa	*Crithidia bombi*	♦	♦	♦	♦	♦		♦	♦	♦			♦					
	Nosema bombi	♦	♦		♦	♦					♦							
	Apicystis bombi	♦	♦	♦	♦	♦	♦											
Parasitoids	*Conopidae*	♦	♦	♦	♦		♦	♦		♦	♦		♦		♦			
	Syntretus splendidus	♦	♦															
Nematode worm	*Sphaerularia bombi*	Q																

Table 4.3 The degree of parasitism by parasites, parasitoids and pathogens of host bumblebee workers and males sampled from the 12 heathlands.

	Dry lowland heathland sites (n=12)			
	mean ± standard error	Median	Range	
			minimum	maximum
Bumblebee Hosts				
Total abundance	1756			
Abundance per site	146.30 ± 13.60	143.50	79.00	223.00
Species richness per site	6.67 ± 0.28	7.00	5.00	8.00
1. Overall Parasitism				
% parasitized (internally or externally)	45.63 ± 1.97	47.36	29.11	54.34
% internally AND externally parasitised	6.13 ± 0.75	6.19	1.37	9.78
2. External mite Parasitism				
Abundance per site	561.00 ± 193.00	351.00	42.00	2531.00
Species richness per site	3.17 ± 0.21	3.00	2.00	4.00
% parasitized externally	24.25 ± 2.32	24.00	10.13	40.46
Mite load per host	3.81 ± 1.48	2.55	0.53	19.77
Mite load per parasitised host	15.06 ± 5.36	9.78	4.53	72.31
3. Internal Parasitism				
Species richness per site	4.25 ± 0.37	4.50	2.00	6.00
% parasitized by > 1 internal species	2.41 ± 0.50	2.06	0.00	5.56
% parasitized internally	27.54 ± 2.78	24.86	12.21	48.94
% parasitized excluding Conopidae	7.93 ± 0.87	8.60	2.53	11.96
a) Conopid parasitism				
% parasitized by Conopidae	22.15 ± 2.82	21.03	6.11	41.84
Conopidae host range	3.92 ± 0.43	4.00	1.00	6.00
Abundance of conopid larvae per site	45.33 ± 8.22	35.50	10.00	93.00
Conopid load per host	0.30 ± 0.04	0.29	0.21	0.37
Conopid load per parasitized host	1.33 ± 0.04	1.31	1.13	1.58
% hosts superparasitized by Conopidae	4.99 ± 1.09	4.56	0.76	15.60
% parasitized hosts superparasitized by Conopidae	21.27 ± 2.36	20.64	6.67	37.29
b) Braconid wasp parasitism				
Abundance of braconid wasp per site	2.50 ± 2.02	0.00	0.00	24.00
% parasitized by braconid wasp	0.14 ± 0.10	0.00	0.00	1.20
Braconid wasp load of hosts	0.01 ± 0.01	0.00	0.00	0.11
Braconid wasp load of parasitized hosts	13.50 ± 10.50	13.50	3.00	24.00
c) Tracheal mite parasitism				
Abundance of tracheal mites per site	140.20 ± 66	67.00	0.00	815.00
% parasitized by tracheal mites	0.97 ± 0.34	0.63	0.00	4.11
Tracheal mite load of hosts	1.34 ± 0.83	0.47	0.00	10.32
Tracheal mite load of parasitized hosts	159.30 ± 84.20	83.00	16.00	815.00
d) Protozoan parasitism				
% parasitized by protozoan(s)	6.76 ± 0.78	7.65	1.27	10.87
% parasitized by *Crithidia bombi*	5.27 ± 0.71	5.66	1.27	8.89
% parasitized by *Nosema bombi*	0.56 ± 0.19	0.33	0.00	2.05
% parasitized by *Apicystis bombi*	0.93 ± 0.22	0.75	0.00	2.17

Table 4.4 The phenology of natural enemies of host bumblebees sampled foraging in dry lowland heathland. For all natural enemies with exception to protozoans, values per sampling session represent the number of parasite individuals sampled from all bumblebee hosts collected for all sites. For the protozoan parasites, values represent the number of host individuals parasitized by these pathogens, as only the presence or absence of these pathogens were recorded.

			Sampling session					
			1 (late June)	2 (mid July)	3 (early Aug)	4 (late Aug)	5 (mid Sept)	Total
Host bumblebee abundance			313	264	384	424	371	1756
Bumblebee natural enemies								
External								
Mite abundance	*Kuzinia spp.*		582	1370	1911	654	1619	6136
	Parasitellus fucorum		161	74	44	27	120	426
	Scutacarus spp.		1	0	7	0	2	10
	Hypoaspis spp.		14	17	42	1	82	156
		Total	758	1461	2004	682	1823	6728
Internal								
Tracheal mite abundance	*Locustacarus buchneri*	egg	0	590	0	440	120	1150
		larviform ind.	0	380	0	75	19	474
		gravid female	0	26	0	25	7	58
		Total	0	996	0	540	146	1682
Nos. of hosts harbouring protozoan(s)	*Crithidia bombi*		38	16	15	15	8	92
	Nosema bombi		1	2	6	2	0	11
	Apicystis bombi		3	4	7	3	0	17
		Total	42	22	28	20	8	120
Parasitoid abundance	Conopidae	egg	3	5	10	12	0	30
		1st instar	15	81	60	48	2	206
		2nd instar	39	41	51	41	6	178
		3rd instar	26	29	17	45	5	122
		puparium	0	7	0	0	0	7
		Total	83	163	138	146	13	543
	Syntretus splendidus	egg	16	0	0	0	0	16
		2nd instar	4	0	0	0	0	4
		4th instar	10	0	0	0	0	10
		Total	30	0	0	0	0	30

Table 4.5 Comparing the prevalence of parasitism between bumblebee host species per caste, using ANCOVA to control for the effect of wing length (used as a proximate measure for host size). Host species included in the analyses are *Bombus lucorum, B. terrestris, B. pascuorum, B. lapidarius* and *B. jonellus*. When analysing workers, *B. lucorum* and *B. terrestris* were combined as *B. luc/terr* due to the difficulty in separating these species. Significant p values (quoted at 95% confidence levels) are indicated by * ($p < 0.05$) and ** ($p < 0.005$).

Caste	parasitism variable	source of variation	degrees of freedom (factor, error)	F	P	significance (95% C.I.)
Workers	Overall parasitism (internal or external)	Host species	1,19	0.81	0.504	
		Mean winglength (Covariate)	1,19	1.45	0.243	
	Internal parasitism	Host species	1,19	5.18	0.009	*
		Mean winglength (Covariate)	1,19	11.24	0.003	**
	External parasitism	Host species	1,19	2.67	0.077	
		Mean winglength (Covariate)	1,19	0.2	0.659	
	Conopid parasitism	Host species	1,19	10.72	<0.001	**
		Mean winglength (Covariate)	1,19	10.92	0.004	**
	Protozoan parasitism	Host species	1,19	1.14	0.358	
		Mean winglength (Covariate)	1,19	3.12	0.093	
Males	Overall parasitism (internal or external)	Host species	1,24	2.91	0.044	*
		Mean winglength (Covariate)	1,24	0.17	0.687	
	Internal parasitism	Host species	1,24	12.04	<0.001	**
		Mean winglength (Covariate)	1,24	5.89	0.023	*
	External parasitism	Host species	1,24	1.99	0.129	
		Mean winglength (Covariate)	1,24	0.09	0.771	
	Conopid parasitism	Host species	1,24	24.11	<0.001	**
		Mean winglength (Covariate)	1,24	2.15	0.156	
	Protozoan parasitism	Host species	1,24	0.67	0.617	
		Mean winglength (Covariate)	1,24	1.13	0.299	

Table 4.6 ANOVA results when comparing the foraging preference of all, unparasitized, and parasitized bumblebees towards flowers of *Erica spp.* and *C. vulgaris* at ancient and restored heathland sites. The mean proportions of foragers found on either flower species are listed together with their standard errors. Bumblebee worker species included in the analyses are *Bombus luc/terr, B. pascuorum, B. lapidarius* and *B. jonellus*. Significant p values (quoted at 95% confidence levels) are indicated by ** ($p < 0.005$).

Heathland type	Parasitism state of bumblebee forager	Factor	D.F. (factor, error)	F	P	significance (95% C.I.)	Mean % foragers on *Erica spp.*	Mean % of foragers on *C. vulgaris*
Ancient sites	all individuals	Flower species	1,10	38.87	<0.001	**	80.12 ± 6.59	19.88 ± 6.59
	unparasitized	Flower species	1,10	32.02	<0.001	**	74.02 ± 5.55	25.98 ± 5.55
	Overall parasitism	Flower species	1,10	41.02	<0.001	**	86.67 ± 8.17	13.33 ± .17
	Internal parasitism	Flower species	1,10	31.28	<0.001	**	84.5 ± 7.97	15.50 ± 7.97
	External parasitism	Flower species	1,10	44.74	<0.001	**	90.33 ± 8.11	9.67 ± 8.11
	Conopid parasitism	Flower species	1,10	26.81	<0.001	**	83.00 ± 8.20	17.17 ± 8.20
	Protozoan parasitism	Flower species	1,10	50.57	<0.001	**	91.17 ± 7.35	8.83 ± 7.35
Restored sites	all individuals	Flower species	1,10	0.20	0.665		52.90 ± 8.46	47.10 ± 8.46
	unparasitized	Flower species	1,10	0.01	0.935		50.78 ± 8.79	49.22 ± 8.79
	Overall parasitism	Flower species	1,10	1.16	0.307		56.50 ± 8.49	43.50 ± 8.49
	Internal parasitism	Flower species	1,10	0.21	0.657		53.00 ± 8.83	47.00 ± 8.83
	External parasitism	Flower species	1,10	3.07	0.11		62.20 ± 10.30	37.80 ± 10.30
	Conopid parasitism	Flower species	1,10	1.10	0.319		42.70 ± 13.40	57.30 ± 13.40
	Protozoan parasitism	Flower species	1,10	14.15	0.004	**	76.50 ± 7.73	23.67 ± 7.73

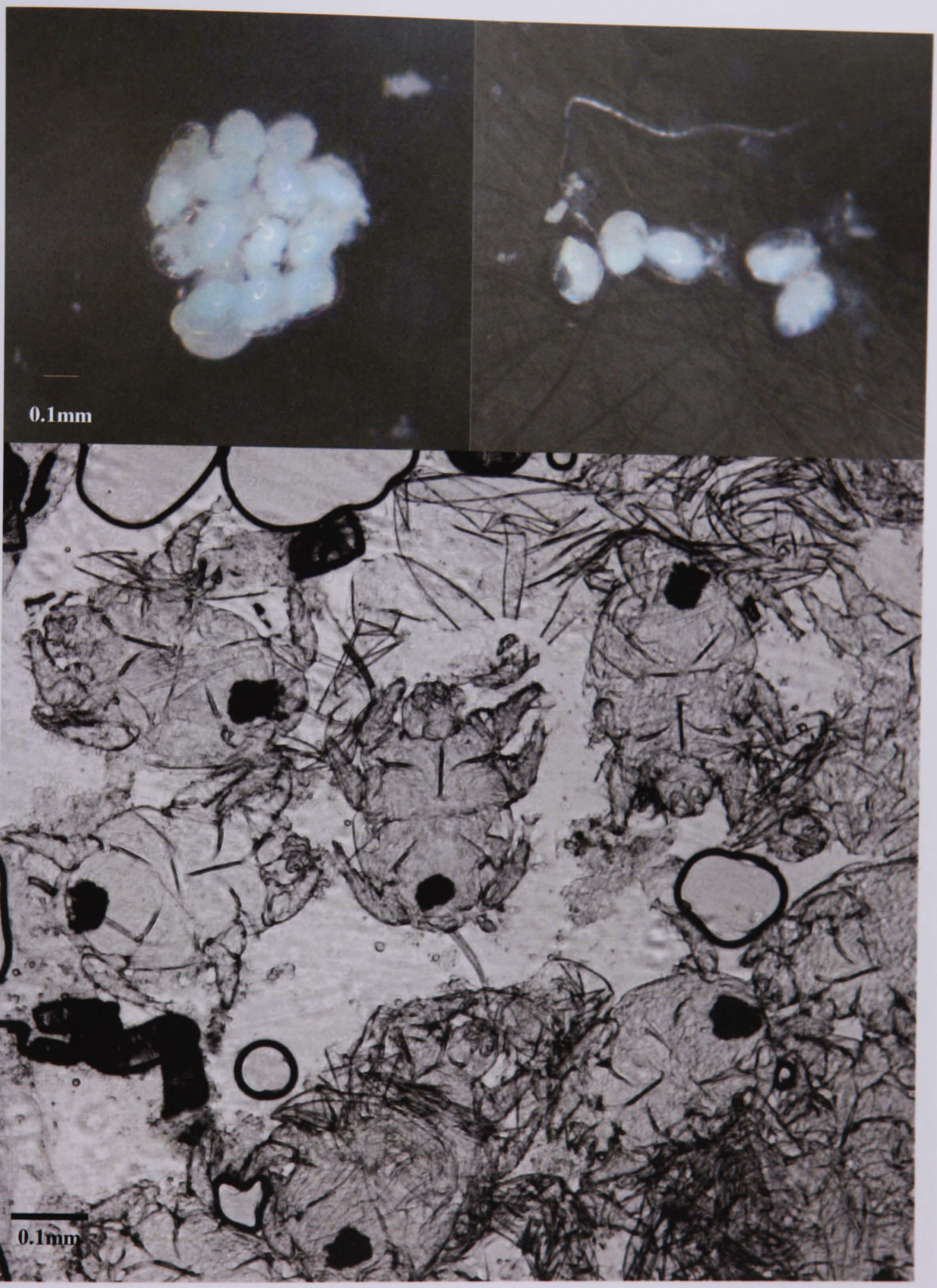


Figure 4.1 The tracheal mite *Locustacarus buchneri,* found in the tracheal system of bumblebee hosts. Top panels: *L. buchneri* eggs; bottom panel: *L. buchneri* larviform females, sampled from bumblebee foragers of heathlands in south Dorset, England.

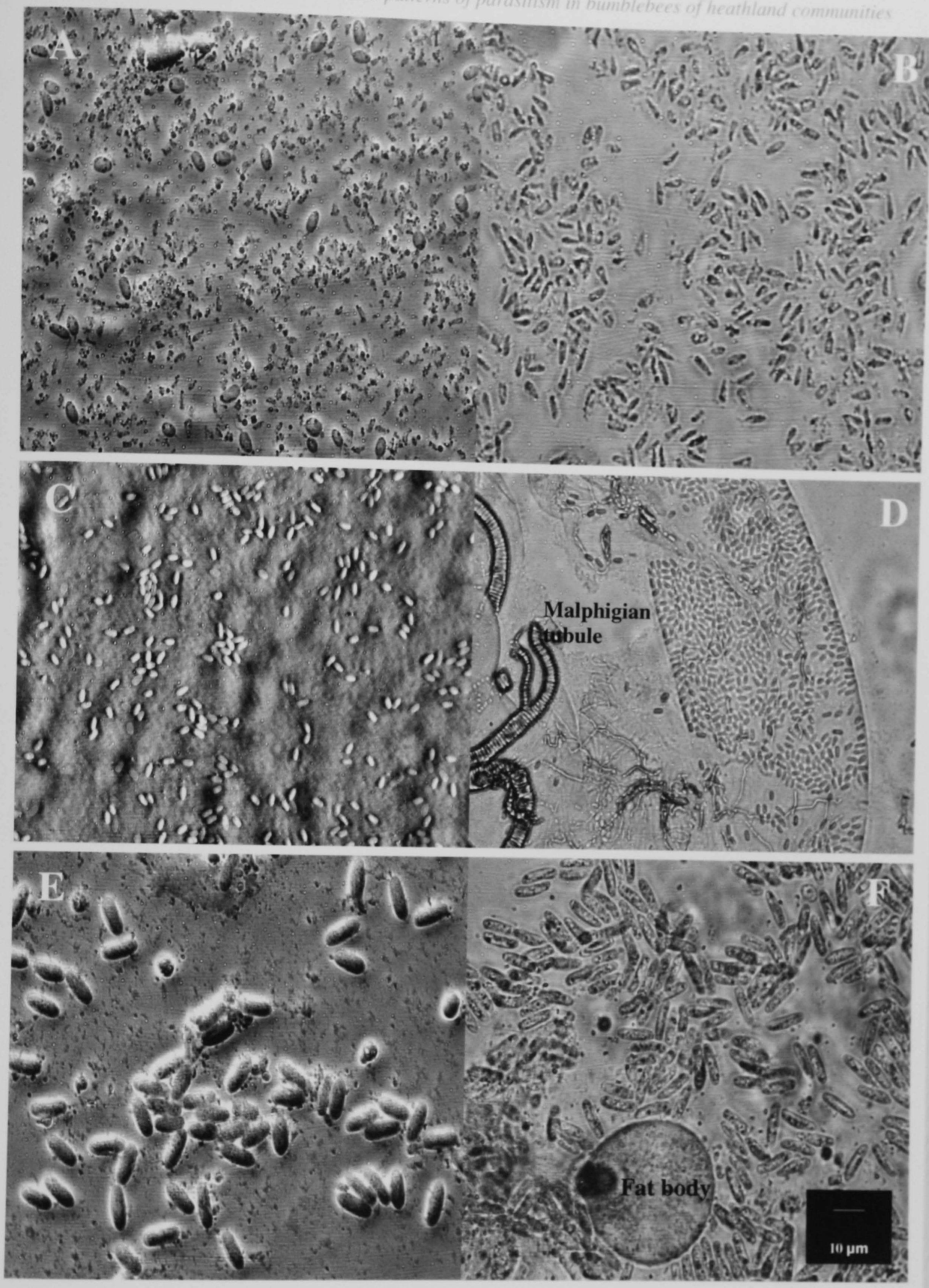


Figure 4.2 Comparative size and morpholohy for three species of bumblebee pathogens sampled from bumblebee foragers in heathlands, south Dorset, England: *Critihidia bombi*, A) motile choanomastigote (with flagellum) and amastigote (without flagellum) forms, B) choanomastigote and promastigote forms following short-term exposure to light under the microscope; *Nosema bombi*, C) spores under phase filter, D) spores in a Malphigian tubule; *Apicystis bombi*, E) sporocysts under phase filter, F) Sporocysts in and around the fat body of its host. Images taken at x400 magnification.

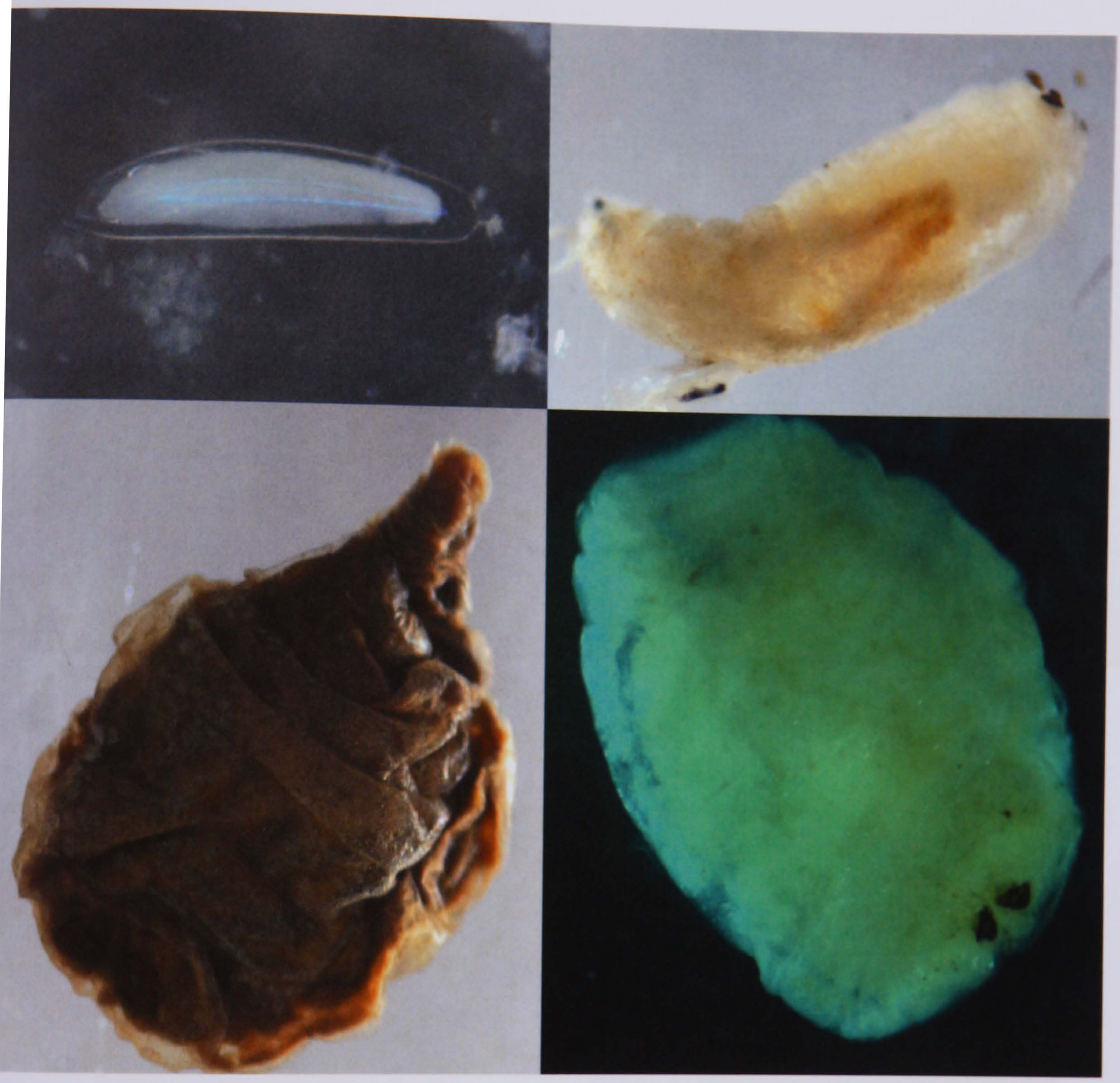


Figure 4.3 Larval stages of Conopidae. Clockwise from top: conopid egg, 2nd instar larvae, 3rd instar larvae, and conopid puparium.

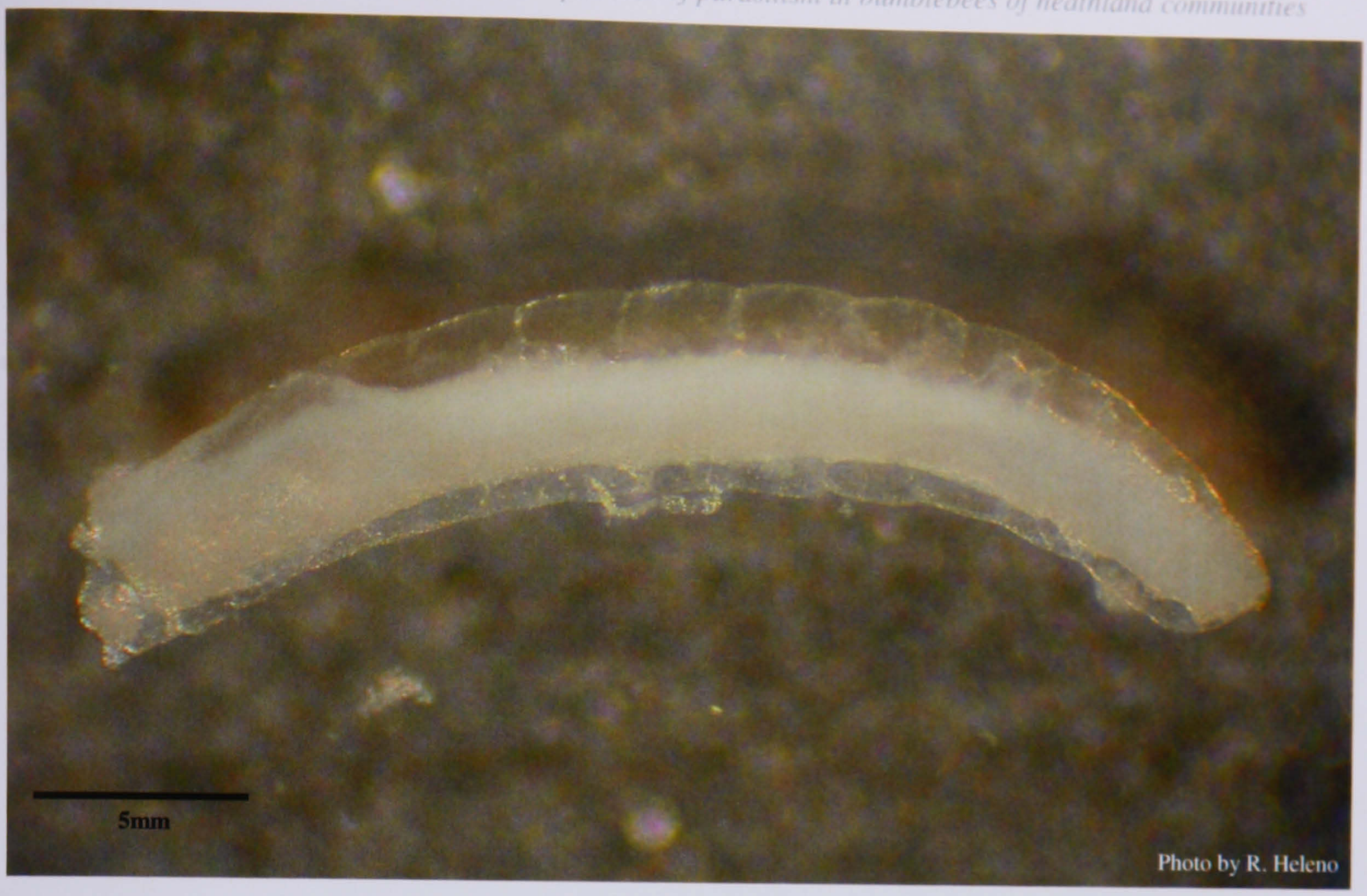


Figure 4.4 Larvae of the braconid wasp, *Syntretus splendidus* (Hymenoptera: Braconidae).

Figure 4.5 Free-moving juveniles of the nematode worm, *Sphaerularia bombi* (Secernentia: Sphaerulariidae).

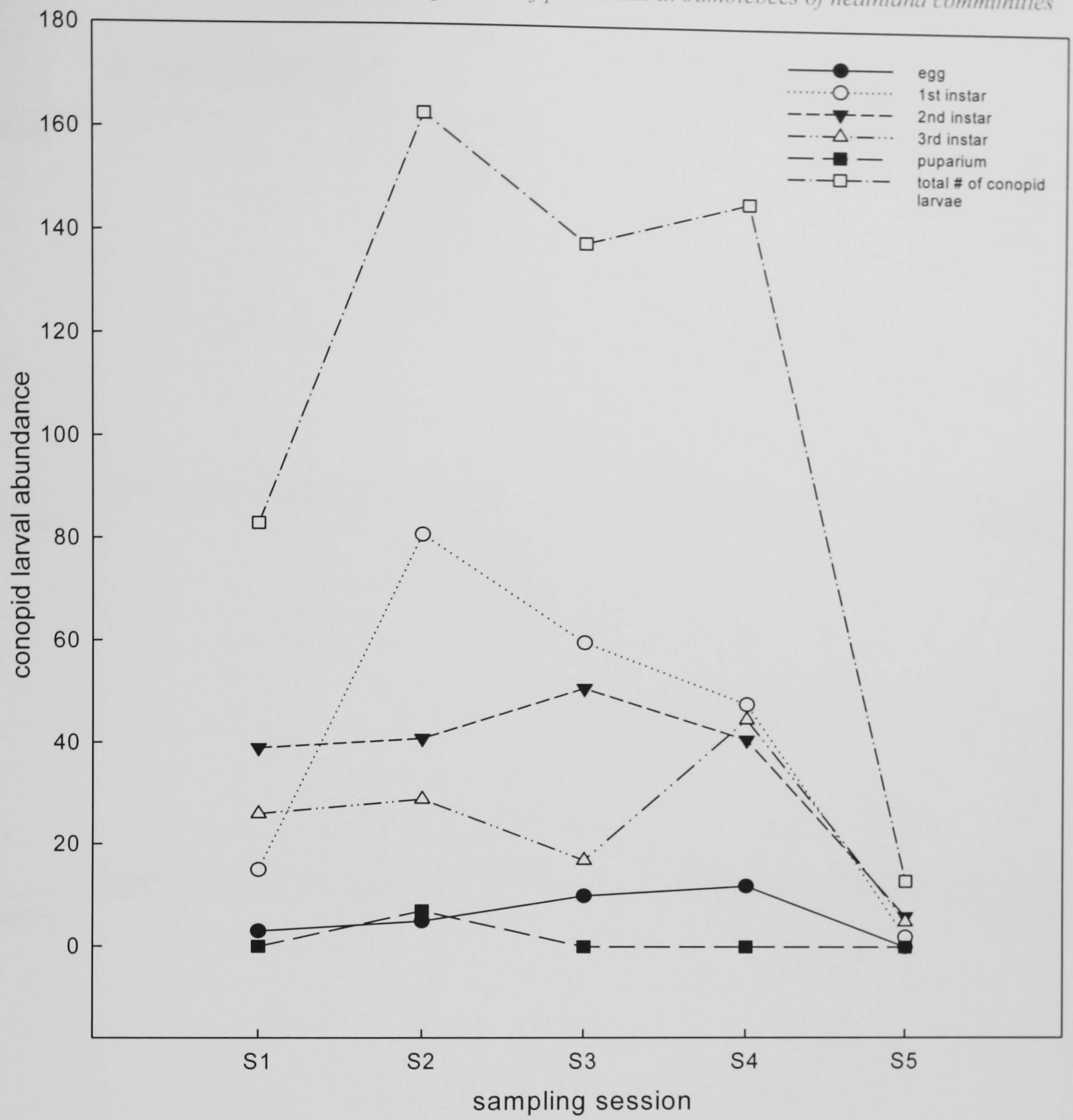


Figure 4.6 The abundance of all conopid larvae found parasitizing *Bombus* hosts collected from heathland. Sampling took place in late June (S1), mid July (S2), early August (S3), late August (S4), and mid September (S5). Abundances are shown for all development stages found in their host: egg, 1st, 2nd and 3rd larval instar, and puparia. Peak abundance of total Conopidae abundance and for 1st larval instar occurred in S2, in S3 for 2nd instar larvae, and in S4 for 3rd instar larvae.

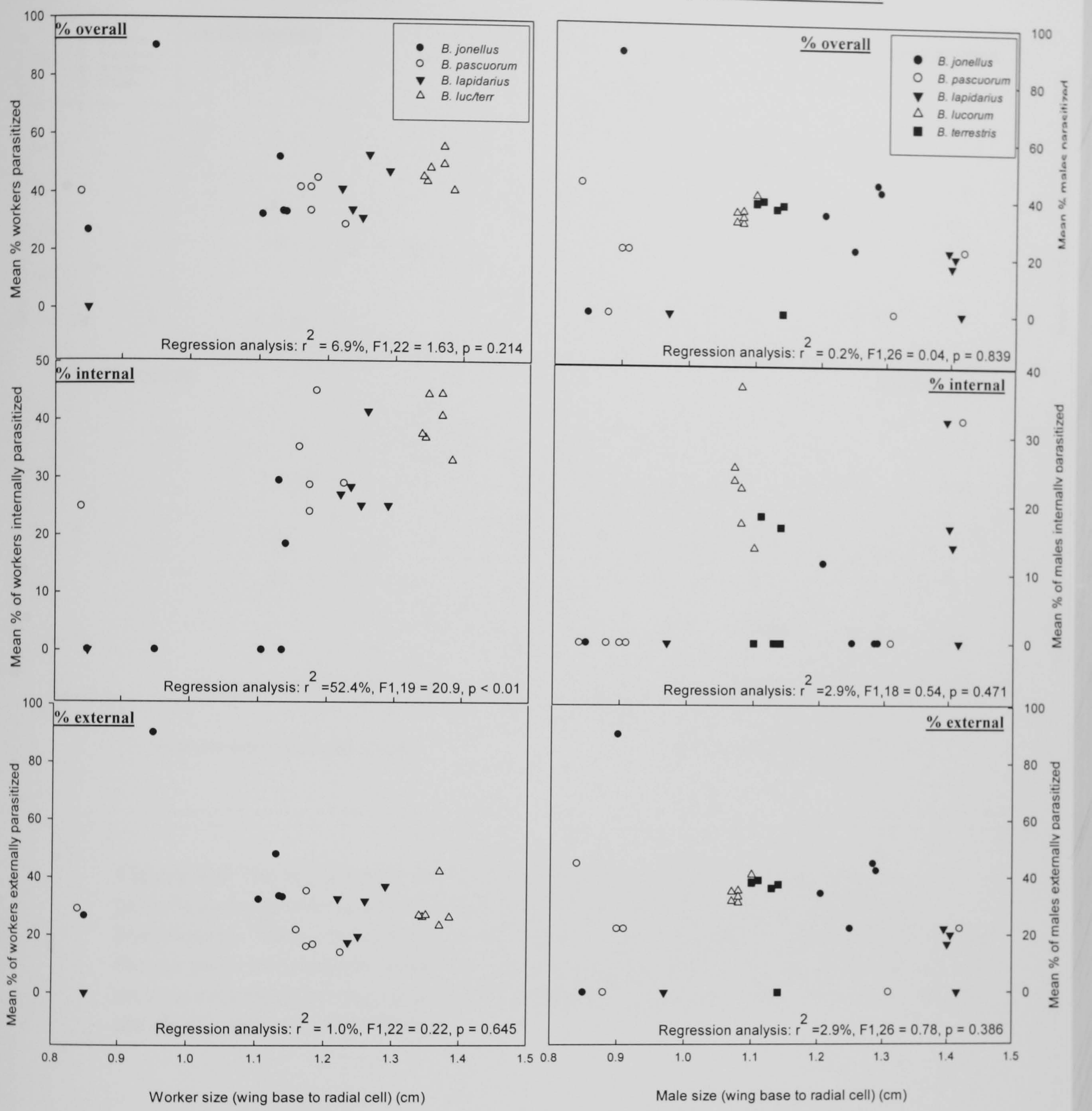


Figure 4.7 The relationship between bumblebee size and the prevalence of overall parasitism, internal parasitism and external parasitism for a) worker bumblebees, and b) male bumblebees. Each point represents the mean value per bumblebee species for each of the six pairs of heathland sampled. The proportions of individuals parasitized are the arcsine-root transformed values of the original proportions. The regression analyses are shown for each relationship.

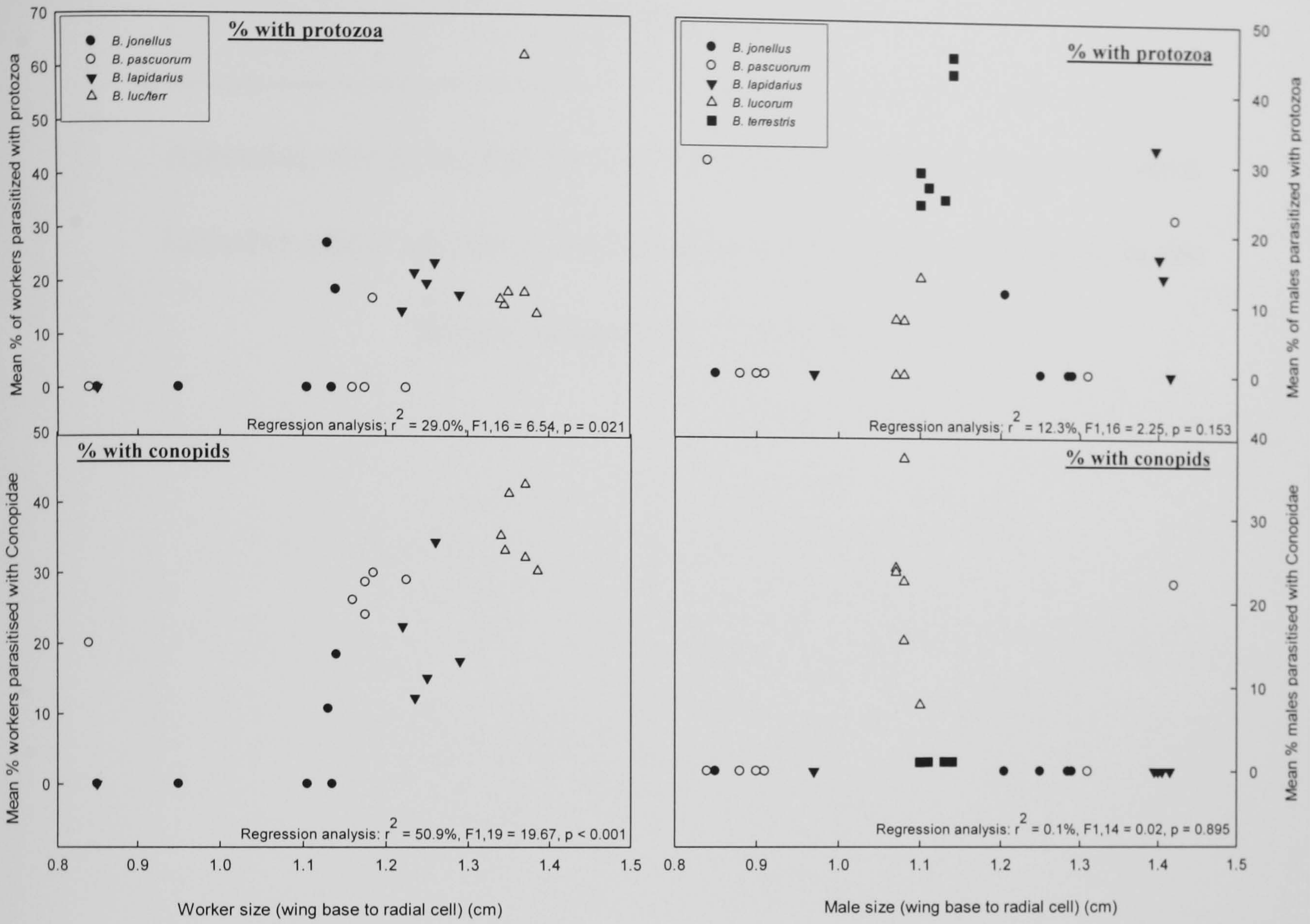


Figure 4.8 The relationship between bumblebee size and the prevalence of parasitism by protozoa and Conopidae for a) worker bumblebees, and b) male bumblebees. Each point represents the mean value per bumblebee species for each of the six pairs of heathland sampled. The proportions of individuals parasitized are the arcsine-root transformed values of the original proportions. The regression analyses are shown for each relationship.

CHAPTER FIVE

Assessing the potential for an "invasional meltdown" by two alien invasive plant species, *Linaria vulgaris* and *Cirsium arvense*, in the Rocky Mountains, Colorado, USA.

CHAPTER FIVE

Assessing the potential for an "invasional meltdown" by two alien invasive plant species, *Linaria vulgaris* and *Cirsium arvense*, in the Rocky Mountains, Colorado, USA.

Summary: *Alien invasive species negatively impact upon communities and ecosystem processes, primarily through interactions such as competition, predation, or herbivory. However, the inclusion of positive interactions in understanding the invasiveness of a species and the invasibility of an ecosystem remains relatively rare. Furthermore, facilitation between alien species themselves is rarely considered, despite increasing evidence for this.*

This study investigated whether facilitative interactions were occurring between two well-established alien plant species, Linaria vulgaris *and* Cirsium arvense, *in the Colorado Rocky Mountains, USA. I assessed their pollinator visitation rates across three sites, where either both alien species co-occurred or where either species occurred alone. Pollen supplementation experiments were carried out to determine pollen limitation, and pollen deposition on the stigmas of both alien species, and their reproductive success with regard to fruit and seed set, were compared between plot treatments.*

I found no evidence for facilitation between Linaria vulgaris *and* Cirsium arvense, *but suggest that due to the pollen limitation of* C. arvense, *management of its spread would benefit from early control. With many alien species historically established, and an increasing number of new invaders spreading globally, positive interactions, whether direct or indirect, are inevitable. Research that increase our understanding of how alien species interact with other species can aid restoration programmes in particular, as restoration must be able to overcome these when either reinstating native species or when removing alien species.*

5.1 Introduction

Biological invasions are considered the second greatest threat to biodiversity after habitat destruction (Wilson 1992; Vitousek *et al.* 1996; Wilcove *et al.* 1998), are globally widespread (Vitousek *et al.* 1997), and are responsible for huge economic losses (Pimentel *et al.* 2001). For example in the USA alone, invasive alien species caused damages and losses valued at 120 billion USD per year (Pimentel *et al.* 2005), whilst in China, losses were equivalent to 1.36% of the GDP (Xu *et al.* 2006). Such detrimental and costly impacts have driven research on biological invasions to focus particularly on understanding which attributes make an invader successful (Rejmanek & Richardson 1996; Williamson & Fitter 1996; Kolar & Lodge 2001; Richardson & Pysek 2006), and which factors predominantly contribute to ecosystem invasibility (Elton 1958; Levine & D'Antonio 1999; Lonsdale 1999; Davis *et al.* 2005). Determining, for example, which characteristics explain the extent of an invader's distribution and abundance, or how factors such as habitat suitability, habitat disturbance, community diversity, and community structure affect community invasibility, can provide a framework for biological invasions from which to predict useful trends and generalities (Rejmanek & Richardson 1996; Lonsdale 1999; Stohlgren *et al.* 1999).

However the use of such theoretical models or short-term experiments in predicting invasiveness and invasibility for a particular species remains questionable (Kareiva 1996; Vitousek *et al.* 1997). This is partially due to huge uncertainties accompanying the invasion process which hinder species-specific predictions (Crawley 1987; Lonsdale 1999; Chapin *et al.* 2000; Sala *et al.* 2000; Thuiller *et al.* 2006). For example, non-linear changes and both synergistic and indirect interactions occur amongst the drivers responsible for ecosystem changes (Sala *et al.* 2000; Simberloff & Gibbons 2004; Mitchell *et al.* 2006; White *et al.* 2006). Drivers include the role of disturbance, resource availability, propagule pressure and competitive release (Lonsdale 1999; Richardson & Pysek 2006). The magnitude and variability of these drivers over both spatial and temporal scales (Richardson *et al.* 1990; Parker *et al.* 1999; Pysek & Hulme 2005; Pauchard & Shea 2006), contribute to the idiosyncratic nature of biological invasions (Richardson *et al.* 1996).

Furthermore the emphasis in the literature has been on negative interactions (i.e. competition) determining community organisation, while positive interactions are rarely considered (Boucher *et al.* 1982; Bertness & Callaway 1994; Richardson *et al.* 2000; Bruno *et al.* 2003). In particular, the theory of biotic resistance (Chapman 1931; Elton 1958) - whereby the biological components of a system such as competitors, predators and parasites, predominantly prevent the establishment of novel alien propagules - has dominated our understanding of community invasibility and has directed conceptual models of community structure (Bertness & Leonard 1997). This theory hypothesises that communities with high species diversity are less invasible due to the reduced number of unoccupied niches and resources available. Although many studies support this (Knops *et al.* 1999; Tilman 1999; Naeem *et al.* 2000), large-scale, observational studies find evidence for the opposite situation in which hotspots of diversity are also highly invaded areas (Robinson *et al.* 1995; Planty-Tabacchi *et al.* 1996; Wiser *et al.* 1998; Levine & D'Antonio 1999; Lonsdale 1999; Stohlgren *et al.* 1999; Ricciardi 2001). They suggest that at larger scales, local negative effects of diversity on invasibility can be overridden by regional, positive effects of habitat heterogeneity and resource availability that promotes both native and alien species (Levine 2000; Byers & Noonburg 2003; Jiang & Morin 2004; Tilman 2004).

The consideration of positive interactions as an influential factor in determining the extent of biological invasions has increasingly been suggested (Boucher *et al.* 1982; Bertness & Callaway 1994; Richardson *et al.* 2000; Bruno *et al.* 2003). Indeed the assimilation of an alien invasive species into the resident community through facilitative interactions can potentially outweigh any competitive resistance to the invasion (Bruno *et al.* 2003; Richardson & Pysek 2006). Their presence could subsequently lead to internal positive feedbacks that promote their persistence, such as their alteration of the disturbance regime (D'Antonio & Vitousek 1992; Hobbs & Huenneke 1992; Chapuis *et al.* 1994), soil communities (Klironomos 2002), or nutrient availability (Vitousek 1990; Von Holle *et al.* 2006), and they can be further facilitated by an array of mutualistic partners such as pollinators and seed dispersers that accumulate over time (Richardson *et al.* 2000).

Furthermore, with the exponential rise in the global spread of alien species over the last 200 years (Lovei 1997; Mack *et al.* 2000; Levine & D'Antonio 2003),

potential facilitative interactions between alien species themselves, whether direct or indirect, are inevitable (Parker *et al.* 1999; Richardson *et al.* 2000; Ricciardi 2001; Grosholz 2005). Mechanisms for the occurrence of such "invader complexes" (D'Antonio & Vitousek 1992 cited by Olesen *et al.* 2002), include the increase in the likelihood of reuniting mutualistic partners in their invaded range (Kaufmann *et al.* 1991; Barthell *et al.* 2001; Adams *et al.* 2003; Diez 2005), or the creation of new mutualisms between alien invasive species that had no coevolved history (Ricciardi 2001; Helms & Vinson 2003; Constible *et al.* 2005). Furthermore, recently introduced invaders have been documented to trigger the acceleration in the expansion of otherwise historically benign invaders through facilitation (Grosholz 2005).

Such facilitative interactions will increase the probability of survival and spread of alien invasive species, allowing communities to become more easily invaded, thereby increasing their magnitude of impact on the native ecosystem (Simberloff & Von Holle 1999). More worryingly though, is the idea that repeated invasions would increase the potential for facilitative interactions to cause positive feedbacks and synergistic effects, where the total impact of an interacting group of alien species far exceeds the sum of their individual impacts on a community (Parker *et al.* 1999) (Simberloff & Von Holle 1999). This "invasional meltdown" process, first investigated by Simberloff and Von Holle (1999), has been documented in coastal (Floerl *et al.* 2004; Grosholz 2005), fresh water (Ricciardi 2001; Adams *et al.* 2003), and terrestrial systems (Helms & Vinson 2003; O'Dowd *et al.* 2003; Parker *et al.* 2006). However evidence on the true population-level impacts of aliens facilitating one another remains scarce, though many of these studies suggest that population-level effects are inevitable (Simberloff 2006).

This study investigates the potential for facilitation between two alien invasive plant species, *Linaria vulgaris* Mill. (common toadflax, Scrophulariaceae), and *Cirsium arvense* L. Scop. (creeping thistle, Asteraceae). Working in the alpine meadows of the Rocky Mountains, Colorado, USA, I tested for the indirect facilitation of either species by the other, with regard to pollinator visitation and subsequent reproductive success. Specifically, I asked: 1) did the abundance, species richness, and rate of visitation by potential pollinators to the floral resources of one target alien species increase when co-occurring with the other alien species? 2) Did

the co-occurrence of both alien species affect the quantity and quality of the pollen load deposited on their stigmas? And 3) was pollen limitation to seed production for either species weakened as a result of their co-occurrence? More generally, this study attempts to assess what potential exists for facilitation between *L. vulgaris* and *C. arvense* in the Colorado Rocky Mountains, and what implications such interactions may have in the management of alien plant species and the restoration of native communities.

5.2 Methods

Study habitat & species

This study was conducted during the summer of 2006 in subalpine meadows located near the Rocky Mountain Biological Laboratory (RMBL) at Gothic, Colorado, U.S.A. (106°59'15" N; 38°57'30" W; 2900m). Surrounding habitat consisted of subalpine fir and spruce forests, aspen and willow, and wildflower meadows. Sampling took place between the 17th of July and 14th of September 2006 during a relatively wet season. Two plant species were selected as the focal alien species for this study: *Linaria vulgaris* Mill. (common toadflax, Scrophulariaceae) and *Cirsium arvense* L. Scop. (creeping thistle, Asteraceae).

Linaria vulgaris is a self-incompatible, perennial, clonal herb, composed of numerous ramets connected underground by short lateral roots. Ramets consist of conspicuous, zygomorphic yellow flowers that flower sequentially upwards starting from the base of the stalk. Flower lifespan is between 3 and 5 days, and each flower is approximately 15-25mm in size with a long nectar-collecting spur extending beyond the base of the flower (Arnold 1982). The lower, orange lip of the corolla acts as a nectar guide, and requires the insects' weight to depress the lower lip away from the upper lip to gain access to pollen and nectar. Small insects that can fit between the lips are also able to access floral rewards. However, nectar robbery is common for this species (Stout *et al.* 2000; Irwin & Maloof 2002), with both primary and secondary robbery occurring in the field. At RMBL, flowering occurred from July until September with peak bloom occurring in mid-August. *L. vulgaris* has not been found to be pollen limited (R.E. Irwin personal communication; Arnold 1982).

Cirsium arvense, a perennial, rhizomatous herb up to 1.5m tall, also has creeping lateral roots that can lead to dense clonal stands. This dioecious thistle produces approximately 40 lilac to white flower heads per flowering shoot, typically 10 – 15mm in diameter with purplish involucral bracts. In the field populations used in this study, shoots of *C. arvense* flowered from August till September. Each head consisted of approximately 100 florets, and produced copious nectar. Male and female flowers are morphologically distinct at all stages of maturity (Lloyd & Myall

1976); most obvious are the visible abundance of pollen in male heads and a strong vanilla fragrance from female heads when receptive (pers. obs). However up to a quarter of male heads have been found to be hermaphrodite, although documented to have very low seed sets (Lloyd & Myall 1976; Kay 1985). These males exhibit a shorter pappus and involucre, and their fruit (achenes) are not released as rapidly as in females upon drying of the involucre ((Lloyd & Myall 1976; Kay 1985). Non-hermaphrodite heads are otherwise obligate outcrossers, relying on insects for pollination as well as a close enough pollen source for successful seed set (Lalonde & Roitberg 1994).

Both *L. vulgaris* and *C. arvense* are native to Europe. As aliens, they are highly invasive and are currently widespread in North America. *L. vulgaris* was introduced to North America in the 1700's as an ornamental, and has since escaped cultivation (Arnold 1982; Mack 2003). Its creeping roots allow it to form large vegetative stands, and, combined with its prolific, winged, seed production, can spread and establish aggressively (Nadeau *et al.* 1991; Saber *et al.* 1995). *L. vulgaris* has proven a significant threat to native biodiversity at both low and high elevation, and in all habitat types, ranging from open, protected areas to disturbed environments (Pauchard *et al.* 2003). *C. arvense* was introduced to North America in the 1600's, spreading as a contaminant in agricultural seeds and manure (Moore 1975); Hansen 1918 cited in (Nuzzo 1997). *C. arvense* has been documented to directly compete and displace native plant species, reduce species diversity and alter the structure and composition of habitats in Colorado (Stachon & Zimdahl 1980). Both species primarily spread through extensive vegetative growth of their roots, and secondarily through copious seed production and establishment.

L. vulgaris and *C. arvense* can tolerate a range of soil types and environmental conditions, invading both agricultural land and natural plant communities, and establishing particularly well at disturbed sites. As such, the United States Department of Agriculture (USDA) lists them both as noxious weeds. Regulating their control of spread however is difficult, and their current North American distributions remain extensive. Indeed *L. vulgaris* seeds are able to lie dormant for at least ten years (Carder 1963), whilst *C. arvense* seeds have been documented to remain viable even up to 21 years in the U.S. (Toole & Brown 1946).

Site selection and plot treatments

Study plots were located across four replicate valley sites between Crested Butte and Gothic in the Colorado Rocky Mountains: Gothic (GT), Lower Loop (LL), Slate River (SR) and Washington Gulch (WG) (Table 5.1). Barriers including mountains, rivers, lakes, and roads separated these sites geographically. All sites are accessible to the public. Due to unforeseen circumstances however, data collection from the study plots at two sites (SR and WG) could not be completed due to extensive damage received midway through the season (including heavy grazing by escaped cows and damage from machinery and trampling). An additional replicate site located on private land nearby, Moon Ridge (MR), was therefore subsequently selected (Table 5.1). Thus three replicate sites were used in this study, as no further suitable replicate sites could be found to increase the number of replicate sites sampled.

Within each replicate site, three 20m x 20m plots of similar elevation were established for this study: *L. vulgaris* plots (hereafter Lv plots) contained only the alien *L. vulgaris; C. arvense* plots (hereafter Ca plots) contained only the alien *C. arvense;* mixed plots contained both *L. vulgaris* and *C. arvense* co-occurring in roughly equal densities within the plot boundary. Any *L. vulgaris* plants flowering near Ca plots were weeded out, up to 50m away from the Ca plot boundary, as were any *C. arvense* plants occurring near Lv plots. Given that these single alien plots were chosen to maximise isolation from the other alien species, weeding was a rare chore. Native flowering plants were present in all plot treatments at all sites. Replicate sites were separated on average by 6.6km, ranging between 2 and 11km (with SR and WG separated by only 2km). Treatment plots within each site were separated by an average of 530m, ranging from 150m to 1km.

Sampling design

A total of 8 sampling sessions were conducted, during which measures of floral abundance and diversity, alien plant flowering densities, insect visitation, fruit and seed set, as well as pollen receipt to stigmas were recorded. At the end of the season,

all alien plants in the treatment plots used were weeded by hand as part of the weed control program at the Rocky Mountain Field Station.

Floral abundance and species richness

To determine the abundance and diversity of floral resources available to insect visitors at our treatment plots over our study season, the number of floral units (a 'unit' being defined as one which an insect flies to rather than walks between) (Dicks *et al.* 2002), and the identity of all flowering species were recorded from within the boundaries of five, 1m^2 quadrat randomly thrown over the treatment plot. Quadrat measurements were taken per treatment plot per week, at all replicate sites.

Alien flowering densities

The total number of *L. vulgaris* and/or *C. arvense* floral units in bloom per plot was approximated for all replicate sites per sampling week. Floral density per m^2 per plot was calculated by dividing this total number of floral units by the total plot area of 400m^2. However as both species existed as patches in the landscape, a second measure of floral density, hereafter referred to as floral density per m^2 per patch, was calculated. This was equated to the total number of floral units in bloom divided by the actual area of coverage by the alien plant species for each plot.

Pollinator visitation

Observations were conducted to assess how many visitors were received at flowers of our target alien species, as well as recording their identity and the number of visits they made, over a set period of time. At each treatment plot per site, four periods of observation per alien plant species were carried out per week. Thus pollinator visitation to *L. vulgaris* was observed at Lv plots and mixed plots, whilst visitation to *C. arvense* was observed at Ca and mixed plots for all sites. Visitation observations were carried out by a single observer standing 1 to 3m from the patch observed, and each observation period lasted 15 minutes. Prior to the commencement of an

observation period, a small flowering patch of the alien species to be observed was chosen at random, and the number of floral units counted. Patch size varied between observation periods, though for *L. vulgaris* plots were frequently less than $1m^2$ in size and containing an average of 150 floral units. For *C. arvense*, patch size was less than $2m^2$, containing an average of 30 flowering heads. All visitors coming into contact with the reproductive organs of a floral unit during the observation were considered potential pollinators and each visit made was recorded. The identity of each visitor and their total number of visits to the observed floral patch were all recorded for each observation period. Observations of nectar robbery by visitors chewing or accessing previously chewed holes without touching the reproductive structures of the flower were similarly recorded, and are hereafter referred to as robbers. Observations were only recorded on dry, warm days between 9am and 6pm throughout the season. Recorded temperatures averaged at 24 °Celsius over the season, ranging between 19 and 26 °Celsius.

Three main measures of visitation rate were calculated from these observations: The number of visits per floral unit per hour, the number of visitors per floral unit per hour, and the number of visiting species per floral unit per hour. These three measures were each divided into the number of *Bombus,* other hymenopteran, syrphid, and other dipteran visitors, as well as the number of visiting *Bombus* species per floral unit per hour. Bumblebees and hummingbirds were both identified to species, while all other visitors were assigned to morphospecies and voucher specimens kept with the collections of the University of Bristol.

Pollen receipt on stigmas

Ten stigmas from flowers of ten randomly selected *L. vulgaris* ramets were collected from Lv and mixed treatment plots at all sites every week. *L. vulgaris* stigmas were collected from individuals whose corolla had wilted. Twenty stigmas from heads of ten randomly selected *C. arvense* shoots were collected from Ca and mixed treatment plots every week. *C. arvense* stigmas that had darkened in colour and were at the end of their receptive stage were collected from flowering heads.

Stigmas of both species were collected on dry days and kept individually in glassine envelopes. Samples were stored in the refrigerator for no more than three

days before being mounted onto slides and stained with fuschin gel (Kearns & Inouye 1993). The stigma and gel were covered using a clean coverslip, ensuring even coverage by the gel. The stigma was then gently squashed to spread the stigmatic surface over the glass and the slide left to dry. Reference slides of *L. vulgaris* and *C. arvense* pollen were made. Slides were examined under a light microscope at 100x, 200x, and 400x magnification for the presence of pollen grains. Pollen were counted and identified as either *L. vulgaris, C. arvense,* or as designated morphospecies according to size and morphology.

Pollen addition

Flowers of *L. vulgaris* and *C. arvense* were supplemented with additional outcross pollen to test if either species were pollen-limited, comparing both fruit and seed set of hand-pollinated plants to control plants that received pollen from visitors only.

Linaria vulgaris: Sixty stalks were selected per treatment plot at all replicate sites for the experiment, with thirty designated as control stalks and thirty designated for manipulation. These stalks were selected over three of the sampling weeks over the season as follows. During the first week of sampling at Lv and mixed plots, twenty stalks close to blooming were randomly selected per plot. Ten stalks were hand-pollinated with outcross pollen, whilst the remaining ten stalks were designated as controls. A further twenty stalks were similarly selected at the third sampling session and again during the fifth sampling session.

Stamens of *L. vulgaris* supporting dry, freshly dehisced anthers were collected into eppendorf tubes from flowers selected at random from donor populations. Donor populations were situated more than 50 m away from the manipulated treatment plot. The lower lips of the corollas on manipulated stalks receiving pollen were gently pressed with the thumb to expose the stigma. A donor anther was then wiped gently across its surface, depositing out-cross pollen. Hand pollination of stigmas of all open flowers from manipulated stalks was carried out twice a week on dry days. Open flowers that were hand-pollinated were lightly marked at the base of its sepals using a paint pen, whilst control stalks were handled only. Fruits on both manipulated and control stalks were allowed to mature for as

long as possible prior to harvesting. Once harvested, the total number of flowers and the number of fruits set per stalk were recorded back at the lab. Due to time constraints, the number of seeds set per fruit was counted for the ten most mature fruits of each stalk only. As a result of predation of fruits by chipmunks, squirrels and birds, only twenty treatment and twenty control plants were randomly chosen to be used in the analyses. The larvae of the curculionid beetle, *Gymnaetron sp.*, a biocontrol agent which feed off the developing and mature seeds within the fruit, were counted to assess the level of predation to *L. vulgaris* in the field.

Cirsium arvense: Due to the later and shorter flowering phenology of *C. arvense*, only thirty female shoots were selected per treatment plot at all replicate sites for the experiment. The flowering heads of fifteen of the shoots were marked for hand-pollination whilst the flowering heads of the remaining fifteen shoots were designated as controls. Male flower heads from a donor population located between 0.2 and 8 km away from treatment plots were collected into a paper bag during the late morning prior to their dehiscence.

Female flowering heads of manipulated shoots at treatment plots received donor male pollen by the thorough brushing of the male anthers from a whole male head over the female head. This was repeated using up to three male heads to ensure adequate pollen deposition onto the female stigmas. Hand-pollination was carried out twice a week on dry days for all manipulated shoots; control shoots were handled only. Flower heads were left to mature in the field before whole shoots were harvested at the end of the season. For each treatment shoot, the number of aborted heads and the number of heads that had set seed (hereafter referred to as fruit set) were counted. The number of achenes produced per head (hereafter referred to as seed set) was counted for ten mature heads per treatment shoot only, and levels predation noted.

Statistical analyses

One-way and Two-way Analyses of Variance (ANOVA) were used throughout the analyses. Where the parametric assumptions of ANOVA were not met, the Sheirer-

Ray-Hare (SRH) extension of the Kruskal-Wallis test for two way-ANOVA designs (Sokal & Rohlf 1995; Dytham 2003) were used instead.

Analyses of Covariance (ANCOVA) using GLM were used to identify differences in visitation rates to the target alien species in question, between *Linaria vulgaris*-only or *Cirsium arvense*-only plots and *L. vulgaris/C. arvense* mixed plots per site. Visitation variables included in the analyses were the number of pollinator visits, number of visitors, number of species, number of *Bombus* species, number of *Bombus* visitors, number of other hymenopteran visitors, number of syrphid visitors, and the number of other dipteran visitors, per floral unit per hour. The number of robbing visitors and the number of robber visitors per floral unit per hour were also included in the analyses for *L. vulgaris*. Mean floral abundance, floral species richness, and both the alien flowering density per plot and per patch per m^2 of the alien invasive species studied, were used as covariates in the analyses to control for differences in background floral variation between plots and sites.

A similar approach was used to determine differences between plot treatment in the number and proportion of conspecific pollen grains found, the number of pollen grains belonging to the other target alien species studied, the number of other heterospecific pollen grains, and the number of other pollen morphospecies present on the stigmas of the target alien species in question. Visitation data and pollen receipt data were averaged per week prior to statistical analyses.

Depending on whether the assumptions of normality were met, either a two-way ANOVA using GLM or the Sheirer-Ray-Hare test was used to test the effect of plot treatment and pollen treatment on the levels of fruit and seed set for each site for both alien species. Fruit and seed predation per site was also analysed for *L. vulgaris*. All residuals were tested for normality and the data checked for homogeneity of variances. Proportional data was angular transformed prior to analyses.

5.3 Results

Floral measures

Floral abundance was significantly different among sites but not between plot treatments (SRH Test: Site: $F_{2,50} = 1.62$, $p = 0.029$; Plot: $F_{2,50} = 6.96$; $p = 0.315$) (Figure 5.1). Species richness was significantly different between sites but did not differ between plot treatment (SRH Test: Site: $F_{2,50} = 0.25$; $p = 0.022$; Plot: $F_{2,50} = 4.82$, $p = 0.771$) (Figure 5.2, Appendix C).

Plot densities of both species and the patch density of *C. arvense* were found to be significantly different between sites. However both the plot and patch flowering density of *L. vulgaris* and *C. arvense* did not differ between plot treatments (*L. vulgaris* density per plot: SRH test: $F_{1,32} = 0.90$; $p=0.313$; per patch: 2-way ANOVA: $F_{1,32} = 0.97$; $p=0.331$ (Figure 5.3 & 5.4). *C. arvense* density per plot: SRH test: $F_{1,33} = 0.03$; $p=0.799$; per patch: $F_{1,33} = 0.10$; $p=0.925$) (Figure 5.5 & 5.6)).

Pollinator visitation

Linaria vulgaris:

A total of 1410 legitimate visitors were recorded visiting *Linaria vulgaris* flowers in all plots sampled. These visitors represented a total of 33 morphospecies, with 1178 visits from 7 species of bumblebee, *B. appositus, B. bifarius, B. californicus, B. flavifrons, B. nevadensis, B. occidentalis,* and *Psithyrus insularis*. 12 other hymenopteran, 7 dipteran, 6 lepidopteran morphospecies and the broad-tailed hummingbird *Selasphorus platycercus,* were also recorded visiting flowers (Appendix C).

The number of visits, species, *Bombus* species, and other hymenopteran visitors per floral unit per hour differed significantly between sites (Refer to table 5.2 for test statistics). However all measures of visitation to *L. vulgaris* were not affected by whether *L. vulgaris* occurred in the absence of, or in mixed densities with, the other alien species *C. arvense*. Specifically, the number of pollinator visits, the number of visitors, the number of species and the number of *Bombus* species per

floral unit per hour, did not differ between Lv or mixed plot treatments (Figure 5.7). Furthermore, when breaking down the visitors into either *Bombus*, other hymenopteran, syrphid, or dipteran visitors, no significant differences were found between plot treatments. Observations of robber visits per floral unit per hour and the number of robbers per floral unit per hour were also not different between Lv and mixed plots (Table 5.2).

Cirsium arvense:

A total of 2707 visits to *C. arvense* from 98 morphospecies were recorded for all plots sampled. 60% of visits were by hymenopterans, with 461 visits from 6 species of *Bombus: B. appositus, B. bifarius, B. californicus, B. flavifrons, B. nevadensis, B. occidentalis,* and *Psithyrus insularis*, and 1147 visits from 33 other hymenopteran morphospecies. A further 1052 visits were made by 43 dipteran morphospecies, the remaining 47 visits to *C. arvense* being from 11 lepidopteran and 4 coleopteran morphospecies and the broad-tailed hummingbird *S. platycercus* (Appendix C).

Again, pollinator visitation rates to flower heads of *C. arvense* were not significantly different when in the presence or absence of the other alien invasive species *L. vulgaris* (Figure 5.8). Specifically the number of visits, visitors, species, *Bombus* species, *Bombus* visitors, other hymenopteran visitors, syrphid visitors, and other dipteran visitors per floral unit per hour did not differ significantly between Ca and mixed plot treatments (Table 5.2). These visitation rates also did not differ significantly between sites sampled, with the exception of the number of other hymenopteran visitors per floral unit per hour, although the level of this significance was small.

Pollen receipt on stigmas

Linaria vulgaris stigmas:

Linaria vulgaris pollen grains were easily identifiable due to their small size (approximately 0.2μm diameter) and relatively simple morphology (Figure 5.9). The total number and proportion of conspecific pollen grains on *L. vulgaris* stigmas

sampled did not differ between sites, or between stigmas sampled from Lv plots and mixed plots (Table 5.3). At the Lv plots, *L. vulgaris* pollen grains represented 99.93% ± 0.0003% of the total number of grains present on *L. vulgaris* stigmas. At mixed plots, *L. vulgaris* pollen grains represented 99.92% ± 0.0003% of the total grains present on stigmas (Figure 5.10). Neither the heterospecific pollen load nor the mean number of heterospecific found on *L. vulgaris* stigmas differed between sites or between Lv and mixed plot treatments (Table 5.3) (Figure 5.11).

Cirsium arvense stigmas:

Cirsium arvense pollen grains have acutely pointed sculpturing elements, and were distinguished from other pollen species by its relatively large size (approximately 0.4µm in diameter) (Figure 5.12). The total number and proportion of conspecific pollen grains found on *C. arvense* stigmas were significantly different between sites and plot treatments (Table 5.3). Greater conspecific loads were found on stigmas sampled from Ca plots than from mixed plots (a mean of 30.63 ± 9.32 *C. arvense* grains representing 53.7% ± 0.11% of grains found per stigma at Ca plots, whilst only 9.25 ± 3.19 grains, representing 27.4% ± 0.06% of grains found per stigma at mixed plots) (Figure 5.13). However this difference could be accounted for by the site MR alone, with almost three times as many conspecific pollen grains found on stigmas at Ca than mixed plots (one-way ANOVA: nos. conspecific pollen grains: $F_{1,6} = 21.85$, $p = 0.003$; proportion of conspecific pollen grains: $F_{1,6} = 16.71$, $p = 0.006$).

The number of *L. vulgaris* or other heterospecific pollen grains found on *C. arvense* stigmas differed significantly between sites, but not between plot treatments (Table 5.3) (Figure 5.14). However there was a trend for stigmas from mixed plots holding greater amounts of *L. vulgaris* pollen. For the site GT this difference was significant (one-way ANOVA: $F_{1,6} = 6.91$, $p = 0.034$).

Pollen addition

Proportion of fruit set:

For each of the sites sampled, pollen supplementation had no significant impact on the proportion of fruit set. The effect of plot treatment on the fruit set for both species was not consistent, with sites responding differently (Figures 5.15 & 5.16). At GT, the proportion of *L. vulgaris* and *C. arvense* fruit set was significantly greater for plants at the mixed plot than at the Lv or Ca plot respectively (SRH test: *L. vulgaris*: $F_{1,76} = 8.21$, $p = 0.007$; *C. arvense*: $F_{1,56} = 17.28$, $p = 0.0002$). However the opposite case was true for LL (SRH test: *L. vulgaris*: $F_{1,76} = 9.52$, $p = 0.003$; *C. arvense*: $F_{1,56} = 7.51$, $p = 0.009$). At MR, no significant difference between plot treatments was found (SRH test: *L. vulgaris*: $F_{1,76} = 1.88$, $p = 0.171$; *C. arvense*: $F_{1,56} = 0.58$, $p = 0.471$).

Seed set:

For *L. vulgaris,* pollen-supplemented plants did not set significantly different amounts of seed to control plants at all sites, reconfirming that *L. vulgaris* is not pollen limited (R.E. Irwin, pers. comm.) (Figure 5.17). In the case of *C. arvense* however, plants that received supplement pollen at two of the three sites set significantly greater amounts of seed than control plants (SRH test: GT: $F_{1,56} = 30.58$, $p = <0.000$; LL: $F_{1,56} = 4.42$, $p = 0.043$) (Figure 5.18). Mean seed set between plot treatments differed significantly for both alien plant species at GT and MR sites, although in opposing directions. At GT, the seed set of both species were greater in mixed plots (*L. vulgaris*: two-way ANOVA: $F_{1,76} = 11.72$, $p = 0.001$. *C. arvense*: SRH test: $F_{1,56} = 29.11$, $p < 0.001$). The reverse was true for MR (*L. vulgaris*: two-way ANOVA: $F_{1,76} = 4.35$, $p = 0.037$; *C. arvense*: SRH test: $F_{1,56} = 10.50$, $p < 0.003$). This greater seed set at MR at the Ca plot for *C. arvense* plants may reflect the significantly greater amount of conspecific pollen that was found deposited on stigmas. For LL, mean seed set of both *L. vulgaris* and *C. arvense* did not differ between plot treatments (*L. vulgaris*: two-way ANOVA: $F_{1,76} = 0.24$, $p = 0.628$. *C. arvense*: SRH test: $F_{1,56} = 2.74$, $p = 0.111$).

Between sites, seed set was significantly different between GT and the other sites for both alien species. *L. vulgaris* seed set per fruit was significantly greater (one-way ANOVA: $F_{2,237} = 60.97$, $p = <0.001$), whilst seed set per *C. arvense* head was significantly lower (Kruskall-Wallis test: $H = 45.19$, d.f. = 2, $p = <0.001$).

Fruit and seed predation:

Aside from grazing by cows, predation of *C. arvense* heads was rare, with only 2 flowering heads being predated over the season. Predation of *L. vulgaris* fruits and seeds was far more common, with a total of 266 fruits predated, representing 11.2% of all fruits examined for predation. For *L. vulgaris*, pollen treatment had no affect on predation levels. However plot treatments did have a significant effect at the sites GT and LL. At GT, predation levels were significantly greater at Lv than at mixed plots (SRH test: nos. predators/fruit: $F_{1,76} = 4.88$, $p = 0.029$; nos. predators/predated fruit: $F_{1,76} = 5.47$, $p = 0.021$). At LL however the opposite was true (SRH test: nos. predators/fruit: $F_{1,76} = 13.87$, $p = 0.001$; nos. predators/predated fruit: $F_{1,76} = 16.41$, $p = 0.0003$) (Figure 5.19).

5.4 Discussion

The co-occurrence of the alien plant species *Linaria vulgaris* and *Cirsium arvense* did not attract a greater number of pollinators or pollinator species to either target plant species, nor did their co-occurrence result in either species receiving a greater number of visits to their floral resources than when occurring alone in the resident community. For *L. vulgaris,* it was evident that this species did not suffer from pollinator limitation. However for *C. arvense,* pollen limitation was a major factor in determining reproductive success with regard to seed set. For the plants occurring alone amongst the resident community, just over half of all pollen grains found on its stigmas were conspecific, whilst for plants co-occurring with *L. vulgaris*, only a quarter of pollen deposited were conspecific pollen grains, with a greater number of *L. vulgaris* pollen grains found on *C. arvense* stigmas. Pollen supplementation experiments confirmed this pollen limitation to seed set of *C. arvense*, with hand-pollinated plants setting significantly greater amounts of seeds than control plants at two of three sites. However whether seed set for either species differed at plots where their populations were mixed or at plots where they occurred alone, remains inconclusive as results were not consistent for all sites. At GT, seed set was greater for both species at mixed plots, whereas at MR seed set was greatest when either alien species occurred alone in the resident community. At LL, seed set did not differ for plants whether in the presence or absence of the other species.

L. vulgaris was not pollen limited, and offered both an abundance and long phenology of floral resources to potential pollinators relative to other native species found in the plot communities. *C. arvense* plants that co-occur with *L. vulgaris* and flower over a much shorter period towards the end of the flowering season, could potentially gain pollinators and reduce their pollen limitation to seed set. However, this study found no differences in pollinator visitation rates to both alien species, when in the presence or absence of each other. Alternatively, the pollen loads found on stigmas from this study suggest that the co-occurrence of *C. arvense* and *L. vulgaris* may actually increase the amount of heterospecific pollen deposited on *C. arvense* stigmas, and thereby depress potential seed set of *C. arvense*. However no trend was found with regard to seed set for either species when co-occurring in

mixed plots or existing alone. This together suggests that neither positive nor negative interactions between these alien invasive species are dominant forces in explaining their reproductive success.

Characteristics that contribute to the success of *L. vulgaris* and *C. arvense* in particular include their ability to reproduce vegetatively, as well as producing copious amounts of seed. They are able to colonise a variety of soil and habitat types at both low and high elevation (Stachon & Zimdahl 1980; Pauchard *et al.* 2003), and have been found to successfully invade agricultural land, native communities, and, in particular, disturbed sites. Furthermore, the long dormancy of both *L. vulgaris* and *C. arvense* seed (Toole & Brown 1946; Carder 1963) can promote their persistence and spread into resident communities. However a limiting factor for *C. arvense* is their depressed seed set as a result of pollen limitation, as evident in this study. Lalonde & Roitberg (1994) also show through manipulation experiments that a close enough pollen source was a major determinant in the successful seed set in female plants. Furthermore, they found that the stigmas of *C. arvense* compensated for distant pollen sources by lengthening their receptive period. Indeed within the area of our study, male *C. arvense* populations were relatively hard to find, and may have been an important factor in understanding the variation in the reproductive success of our female *C. arvense* populations.

The relatively small spatial and temporal scales of this work limit the conclusions regarding the potential for facilitation between the two alien invasive plant species, *L. vulgaris* and *C. arvense*. Crucially, the number of replicate sites used in this study was reduced, as two replicate sites were lost to grazing and trampling pressures early on in the season, with only one site found to be a suitable replacement. However despite the small number of replicates used, significant differences between sites were still picked up in the analyses. Finally, as with many short-term studies, replicating this study over more than one flowering season would have been beneficial, although unfortunately logistically unfeasible in the current project.

The mechanisms underlying the spread of alien invasive plants into native resident communities have been relatively well studied (Bond 1994; Rejmanek & Richardson 1996; Richardson & Pysek 2006). Indeed the phenomenon that most pollinators are in fact generalist rather than specialist with regard to their host (Waser

et al. 1996; Waser & Ollerton 2006), allows for pollinator-plant mutualisms to be re-established. Alien invasive plants species thereby easily integrate into their resident native community (Richardson *et al.* 2000; Memmott & Waser 2002; Morales & Aizen 2006). Native communities are therefore susceptible to alien invasive species as a result of these readily acquired mutualisms, with pollinator limitation rarely hindering the spread of alien plants (Bond 1994). This is exemplified by *L. vulgaris*, and possibly by *C. arvense* as the high rates of visitation received by *C. arvense* suggests that pollen limitation was more likely due to low availability of a male pollen source rather than inadequate pollinators.

Our study suggests that the current spread of *C. arvense* may be predominantly due to clonal reproduction. Further expansion of *C. arvense* populations however will increase male abundance and pollen availability, leading to substantial increases in female seed set, thereby enhancing further establishment. This positive feedback may inevitably lead to a substantially greater rate of *C. arvense* spread than currently experienced from vegetative reproduction, with greater implications for the native resident community. Furthermore, conceptual models in biological weed control suppose at least a 60% reduction in seed set before any effective reduction in a weed population (Hoffmann 1990; Shea & Kelly 1998; Richardson *et al.* 2000a). However predation rates to *C. arvense* were negligible, whilst only 11.2% of *L. vulgaris* fruits suffered predation. Any reduction in abundance from predation will therefore be minimal at best.

Although much of predicting biological invasions is speculative, the increasing amount of evidence for positive interactions and synergisms between alien invasive species in the literature supports the need for the management of invasive species to take a more prudent approach. Any future economic costs and impacts upon biodiversity as a result of the build up of invader complexes and invasional meltdowns will likely far outweigh the more minimal cost of their current control. Furthermore, the vast majority of restoration projects typically involve either the removal or management of alien invasive species and their spread (D'Antonio & Chambers 2006). Thus if we are to succeed in restoring habitats, such processes must be adequately understood such that restoration programmes can be designed to overcome these interactions when reinstating native species, or indeed when removing alien ones. Although this study found no evidence for facilitation, the call

for studies to investigate both positive as well as negative interactions between alien and native species, as well as between potentially interacting alien species themselves, remains urgent.

5.5 Tables & Figures

Table 5.1 Study sites were located across replicate valley sites between Crested Butte and Gothic in the Colorado Rocky Mountains: Gothic (GT), Lower Loop (LL), Moon Ridge (MR), Slate River (SR) and Washington Gulch (WG). At each site, three plot treatments were studied: plots where *Cirsium arvense* plants were present and *Linaria vulgaris* absent (Ca), plots where *L. vulgaris* plants were present and *C. arvense* plants were absent (Lv), and mixed plots where both alien species co-occurred in relatively equal densities (Mixed).

Valley	Plot treatment	Elevation (feet)	Longitude	Latitude
Gothic (GT)	Ca Lv Mixed	9502	38°57'33.1"	106°59'20.2"
Lower Loop (LL)	Ca	8997	38°53'56.2"	107°00'59.9"
	Lv	8998	38°54'12.0"38	107°01'25.7"
	Mixed	8968	°53'58.5"	107°01'073"
Moon Ridge (MR)	Ca	9035	38°53'02.9"	106°58'18.3"
	Lv	8905	38°52'47.9"	106°58'29.9"
	Mixed	9029	38°53'00.3"	106°58'23.0"
Slate River (SR)	Ca	8976	38°53'20.8"	106°59'43.8"
	Lv	8987	38°53'13.7"	106°59'16.3"
	Mixed	8946	38°53'07.3"	106°59'11.0"
Washington Gulch (WG)	Ca	9293	38°53'53.8"	106°58'55.4"
	Lv	9382	38°53'42.5"	106°58'49.7"
	Mixed	9372	38°53'44.1"	106°58'39.9"

Table 5.2 ANCOVA results when comparing visitation rates per floral unit per hour at *L. vulgaris* and *C. arvense* between Lv, Ca and mixed plot treatments and sites sampled.

Linaria vulgaris					
variable per floral unit per hour	source	DF (Factor, error)	F	P	sig. at 95% C.I
nos. visits	covariate 1	1,28	3.67	0.066	ns
	covariate 2	1,28	0.29	0.596	ns
	covariate 3	1,28	0.03	0.855	ns
	covariate 4	1,28	0.43	0.517	ns
	site	2,28	3.69	0.038	*
	plot treatment	1,28	0.01	0.909	ns
	site*plot treatment	2,28	0.51	0.609	ns
nos. visitors	covariate 1	1,28	1.17	0.288	ns
	covariate 2	1,28	0.19	0.665	ns
	covariate 3	1,28	0.00	0.999	ns
	covariate 4	1,28	0.07	0.794	ns
	site	2,28	2.49	0.101	ns
	plot treatment	1,28	0.00	0.995	ns
	site*plot treatment	2,28	2.21	0.129	ns
nos. species	covariate 1	1,28	0.50	0.484	ns
	covariate 2	1,28	2.16	0.153	ns
	covariate 3	1,28	0.25	0.618	ns
	covariate 4	1,28	0.21	0.649	ns
	site	2,28	6.41	0.005	*
	plot treatment	1,28	0.90	0.351	ns
	site*plot treatment	2,28	0.06	0.940	ns
nos. *Bombus* species	covariate 1	1,28	0.48	0.493	ns
	covariate 2	1,28	1.21	0.281	ns
	covariate 3	1,28	0.00	0.952	ns
	covariate 4	1,28	1.42	0.244	ns
	site	2,28	5.85	0.008	*
	plot treatment	1,28	1.30	0.265	ns
	site*plot treatment	2,28	1.78	0.187	ns
nos. *Bombus* visitors	covariate 1	1,28	0.37	0.546	ns
	covariate 2	1,28	0.26	0.614	ns
	covariate 3	1,28	0.02	0.892	ns
	covariate 4	1,28	0.05	0.830	ns
	site	2,28	2.16	0.135	ns
	plot treatment	1,28	0.24	0.628	ns
	site*plot treatment	2,28	1.61	0.218	ns
nos. other hymenopteran visitors	covariate 1	1,28	3.22	0.084	ns
	covariate 2	1,28	0.18	0.675	ns
	covariate 3	1,28	0.75	0.395	ns
	covariate 4	1,28	0.49	0.492	ns
	site	2,28	5.99	0.007	*
	plot treatment	1,28	0.14	0.714	ns
	site*plot treatment	2,28	5.72	0.008	*
nos. syrphid visitors	covariate 1	1,28	0.38	0.541	ns
	covariate 2	1,28	3.60	0.068	ns
	covariate 3	1,28	0.31	0.582	ns
	covariate 4	1,28	0.13	0.720	ns
	site	2,28	0.89	0.420	ns
	plot treatment	1,28	0.70	0.411	ns
	site*plot treatment	2,28	0.26	0.772	ns
nos. other dipteran visitors	covariate 1	1,28	0.04	0.852	ns
	covariate 2	1,28	0.17	0.687	ns
	covariate 3	1,28	0.01	0.912	ns
	covariate 4	1,28	0.11	0.747	ns
	site	2,28	0.46	0.637	ns
	plot treatment	1,28	0.61	0.442	ns
	site*plot treatment	2,28	0.41	0.667	ns
nos. robber visitors	covariate 1	1,28	0.85	0.363	ns
	covariate 2	1,28	0.67	0.421	ns
	covariate 3	1,28	0.03	0.859	ns
	covariate 4	1,28	3.97	0.056	ns
	site	2,28	2.94	0.069	ns
	plot treatment	1,28	0.02	0.887	ns
	site*plot treatment	2,28	0.22	0.805	ns
nos. robbing visits	covariate 1	1,28	0.77	0.388	ns
	covariate 2	1,28	1.05	0.314	ns
	covariate 3	1,28	0.00	0.977	ns
	covariate 4	1,28	2.42	0.131	ns
	site	2,28	1.96	0.159	ns
	plot treatment	1,28	0.06	0.814	ns
	site*plot treatment	2,28	0.10	0.909	ns

Circium arvense					
variable per floral unit per hour	source	DF (Factor, error)	F	P	sig. at 95% C.I
nos. visits	covariate 1	1,18	0.14	0.712	ns
	covariate 2	1,18	0.51	0.483	ns
	covariate 3	1,18	0.50	0.490	ns
	covariate 4	1,18	1.67	0.213	ns
	site	2,18	1.64	0.222	ns
	plot treatment	1,18	2.63	0.122	ns
	site*plot treatment	2,18	0.82	0.455	ns
nos. visitors	covariate 1	1,18	0.01	0.925	ns
	covariate 2	1,18	0.95	0.344	ns
	covariate 3	1,18	0.32	0.581	ns
	covariate 4	1,18	0.84	0.371	ns
	site	2,18	69.00	0.513	ns
	plot treatment	1,18	0.72	0.406	ns
	site*plot treatment	2,18	1.06	0.367	ns
nos. species	covariate 1	1,18	0.68	0.421	ns
	covariate 2	1,18	2.61	0.123	ns
	covariate 3	1,18	0.50	0.489	ns
	covariate 4	1,18	0.00	0.991	ns
	site	2,18	1.35	0.285	ns
	plot treatment	1,18	0.24	0.632	ns
	site*plot treatment	2,18	0.53	0.598	ns
nos. *Bombus* species	covariate 1	1,18	0.51	0.485	ns
	covariate 2	1,18	0.84	0.372	ns
	covariate 3	1,18	0.31	0.587	ns
	covariate 4	1,18	0.83	0.373	ns
	site	2,18	1.72	0.207	ns
	plot treatment	1,18	0.87	0.363	ns
	site*plot treatment	2,18	0.30	0.746	ns
nos. *Bombus* visitors	covariate 1	1,18	0.22	0.648	ns
	covariate 2	1,18	0.54	0.472	ns
	covariate 3	1,18	0.00	0.949	ns
	covariate 4	1,18	2.01	0.173	ns
	site	2,18	1.08	0.359	ns
	plot treatment	1,18	1.38	0.255	ns
	site*plot treatment	2,18	0.65	0.532	ns
nos. other hymenopteran visitors	covariate 1	1,18	0.00	0.992	ns
	covariate 2	1,18	4.69	0.044	*
	covariate 3	1,18	0.24	0.628	ns
	covariate 4	1,18	0.26	0.615	ns
	site	2,18	3.63	0.048	*
	plot treatment	1,18	0.69	0.418	ns
	site*plot treatment	2,18	0.93	0.414	ns
nos. syrphid visitors	covariate 1	1,18	0.12	0.734	ns
	covariate 2	1,18	1.37	0.257	ns
	covariate 3	1,18	0.12	0.730	ns
	covariate 4	1,18	.2.67	0.120	ns
	site	2,18	0.19	0.828	ns
	plot treatment	1,18	.2.13	0.161	ns
	site*plot treatment	2,18	1.64	0.222	ns
nos. other dipteran visitors	covariate 1	1,18	0.05	0.831	ns
	covariate 2	1,18	0.16	0.697	ns
	covariate 3	1,18	0.03	0.874	ns
	covariate 4	1,18	1.63	0.216	ns
	site	2,18	0.47	0.632	ns
	plot treatment	1,18	0.00	0.987	ns
	site*plot treatment	2,18	0.60	0.560	ns

covariate 1: Floral abundance per m2

covariate 2: Number of flowering species per m2

covariate 3: Alien density per m2 per plot (averaged over whole plot area (400m2))

covariate 4: Alien density per m2 per patch (averaged over actual area of coverage)

Table 5.3. ANCOVA results when comparing the amounts of *L. vulgaris* pollen, *C. arvense* pollen, and other heterospecific pollen loads and pollen species found on the stigmas of *L. vulgaris* and *C. arvense*, between Lv, Ca and mixed plots for all three sites sampled.

Linaria vulgaris					
pollen variable	**source**	**DF (factor, error)**	**F**	**P**	**sig. at 95% C.I.**
nos. *L. vulgaris* pollen grains	covariate 1	1,28	2.30	0.141	ns
	covariate 2	1,28	4.63	0.040	*
	covariate 3	1,28	4.48	0.043	*
	covariate 4	1,28	4.17	0.051	*
	site	2,28	2.24	0.125	ns
	plot treatment	1,28	1.06	0.313	ns
	site*plot treatment	2,28	0.24	0.788	ns
nos. *C. arvense* pollen grains	covariate 1	1,28	0.90	0.352	ns
	covariate 2	1,28	0.45	0.506	ns
	covariate 3	1,28	0.20	0.892	ns
	covariate 4	1,28	0.10	0.755	ns
	site	2,28	0.75	0.483	ns
	plot treatment	1,28	0.04	0.839	ns
	site*plot treatment	2,28	1.17	0.324	ns
nos. other heterospecific pollen grains	covariate 1	1,28	0.34	0.563	ns
	covariate 2	1,28	2.39	0.134	ns
	covariate 3	1,28	0.01	0.913	ns
	covariate 4	1,28	0.03	0.869	ns
	site	2,28	0.03	0.972	ns
	plot treatment	1,28	0.05	0.819	ns
	site*plot treatment	2,28	0.09	0.918	ns
nos. other heterospecific pollen morphospecies	covariate 1	1,28	1.63	0.212	ns
	covariate 2	1,28	0.31	0.583	ns
	covariate 3	1,28	0.79	0.382	ns
	covariate 4	1,28	0.09	0.765	ns
	site	2,28	1.15	0.332	ns
	plot treatment	1,28	0.02	0.899	ns
	site*plot treatment	2,28	0.51	0.605	ns
proportion of conspecific pollen grains	covariate 1	1,28	0.09	0.769	ns
	covariate 2	1,28	1.17	0.289	ns
	covariate 3	1,28	0.02	0.898	ns
	covariate 4	1,28	0.04	0.851	ns
	site	2,28	0.22	0.804	ns
	plot treatment	1,28	0.00	0.970	ns
	site*plot treatment	2,28	0.15	0.866	ns

Cirsium arvense					
pollen variable	**source**	**DF (factor, error)**	**F**	**P**	**sig. at 95% C.I.**
nos. *L. vulgaris* pollen grains	covariate 1	1,16	0.00	0.986	ns
	covariate 2	1,16	6.34	0.023	*
	covariate 3	1,16	9.23	0.008	*
	covariate 4	1,16	0.50	0.490	ns
	site	2,16	4.61	0.026	*
	plot treatment	1,16	2.86	0.110	ns
	site*plot treatment	2,16	1.08	0.363	ns
nos. *C. arvense* pollen grains	covariate 1	1,16	0.11	0.748	ns
	covariate 2	1,16	0.04	0.835	ns
	covariate 3	1,16	0.01	0.935	ns
	covariate 4	1,16	0.65	0.434	ns
	site	2,16	21.70	0.000	*
	plot treatment	1,16	10.17	0.006	*
	site*plot treatment	2,16	6.00	0.011	*
nos. other heterospecific pollen grains	covariate 1	1,16	5.00	0.040	*
	covariate 2	1,16	16.40	0.001	*
	covariate 3	1,16	19.29	0.000	*
	covariate 4	1,16	4.92	0.041	*
	site	2,16	18.13	0.000	*
	plot treatment	1,16	3.95	0.064	ns
	site*plot treatment	2,16	2.19	0.144	ns
nos. other heterospecific pollen morphospecies	covariate 1	1,16	0.11	0.747	ns
	covariate 2	1,16	4.00	0.063	ns
	covariate 3	1,16	10.03	0.006	*
	covariate 4	1,16	0.01	0.906	ns
	site	2,16	3.83	0.044	*
	plot treatment	1,16	0.16	0.692	ns
	site*plot treatment	2,16	1.16	0.339	ns
proportion of conspecific pollen grains	covariate 1	1,16	0.02	0.889	ns
	covariate 2	1,16	2.01	0.175	ns
	covariate 3	1,16	1.64	0.219	ns
	covariate 4	1,16	0.13	0.718	ns
	site	2,16	18.44	0.000	*
	plot treatment	1,16	6.00	0.026	*
	site*plot treatment	2,16	2.24	0.139	ns

covariate 1: floral abundance per m2
covariate 2: Number of flowering species per m2
covariate 3: Alien density per m2 per plot (averaged over whole plot area (400m2))
covariate 4: Alien density per m2 per patch (averaged over actual area of coverage)

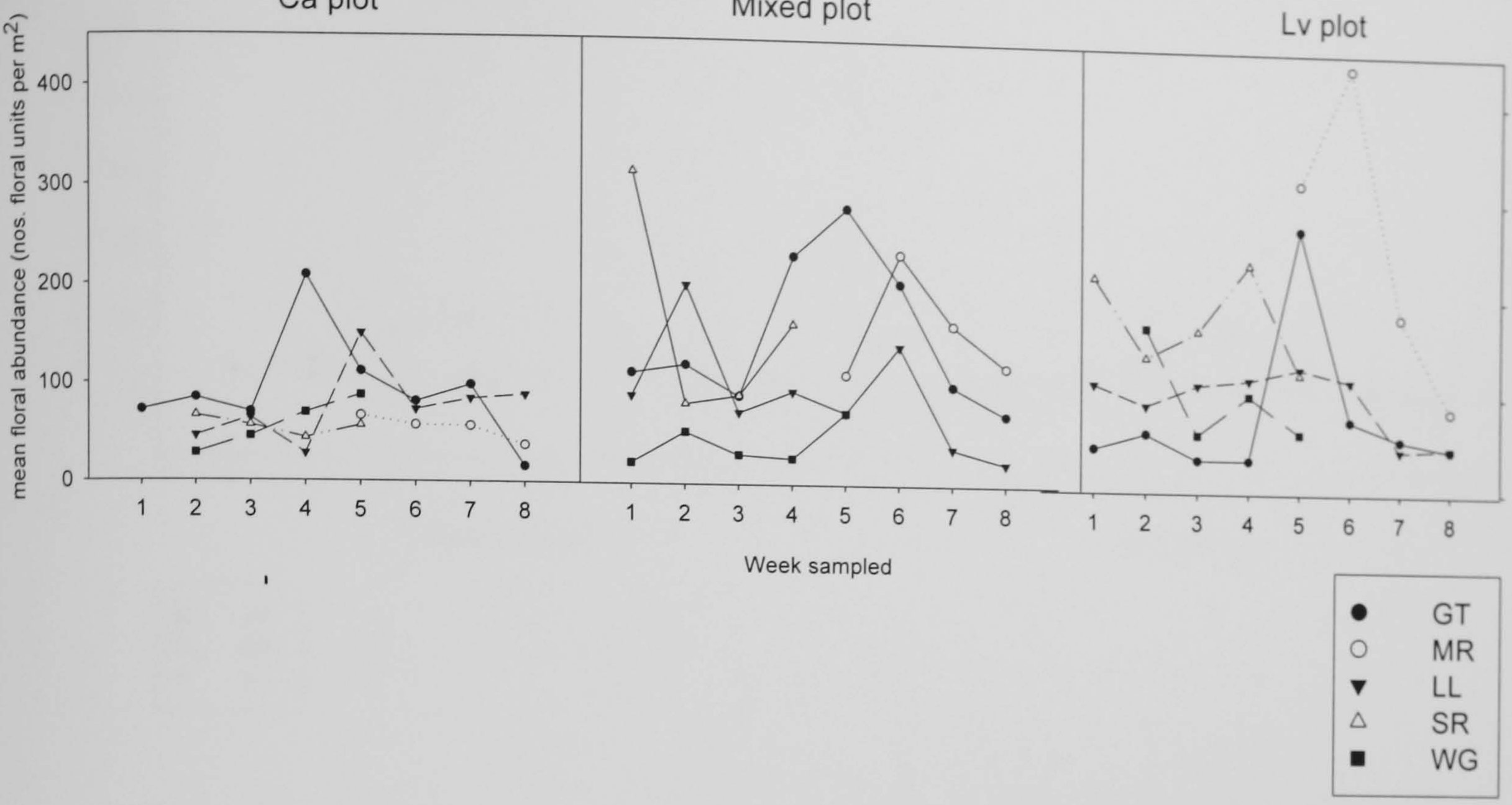


Figure 5.1 Mean floral abundance (number of floral units per m^2) for all five sites sampled over the season per plot treatment. Sampling occurred each week over eight weeks, from mid July (sampling week 1) to mid September (sampling week eight).

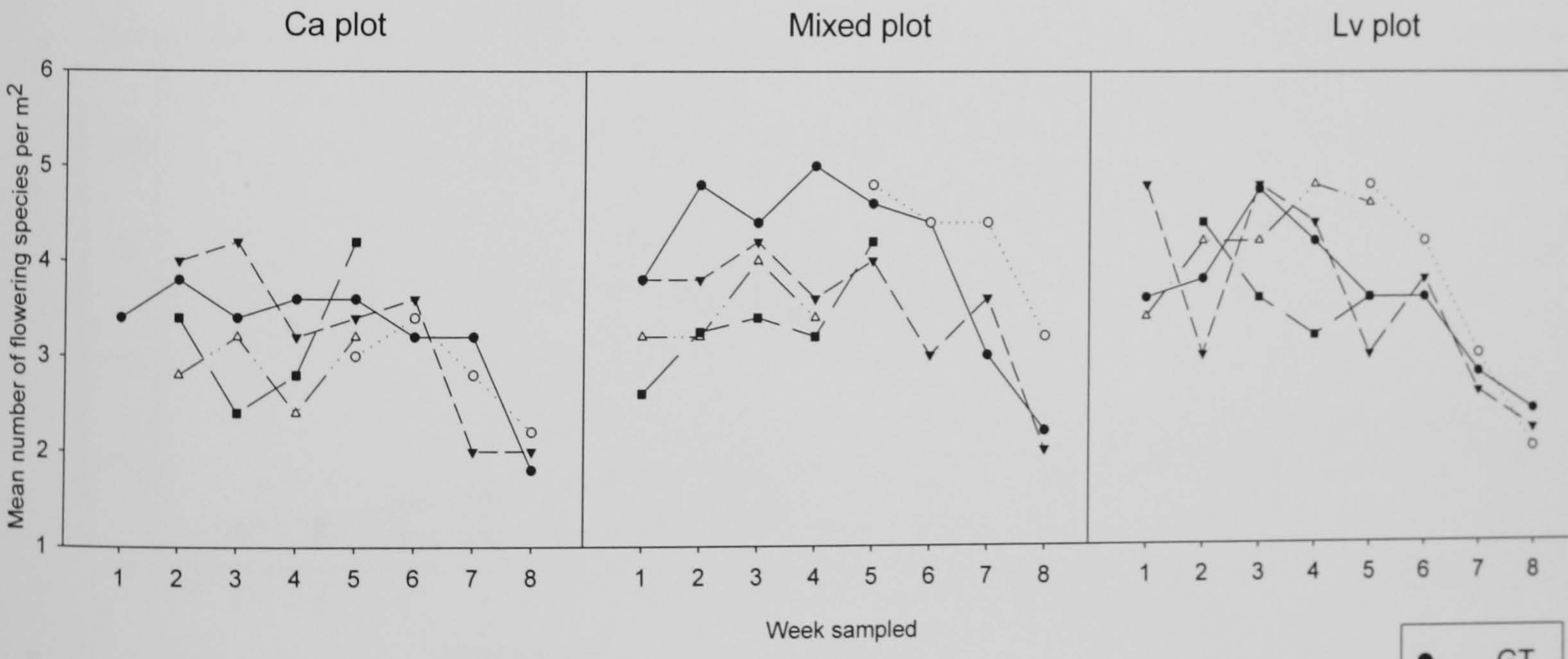


Figure 5.2 Mean number of flowering species per m^2 per plot per treatment at all sites over the season. Sampling occurred each week over eight weeks, from mid July (sampling week 1) to mid September (sampling week eight).

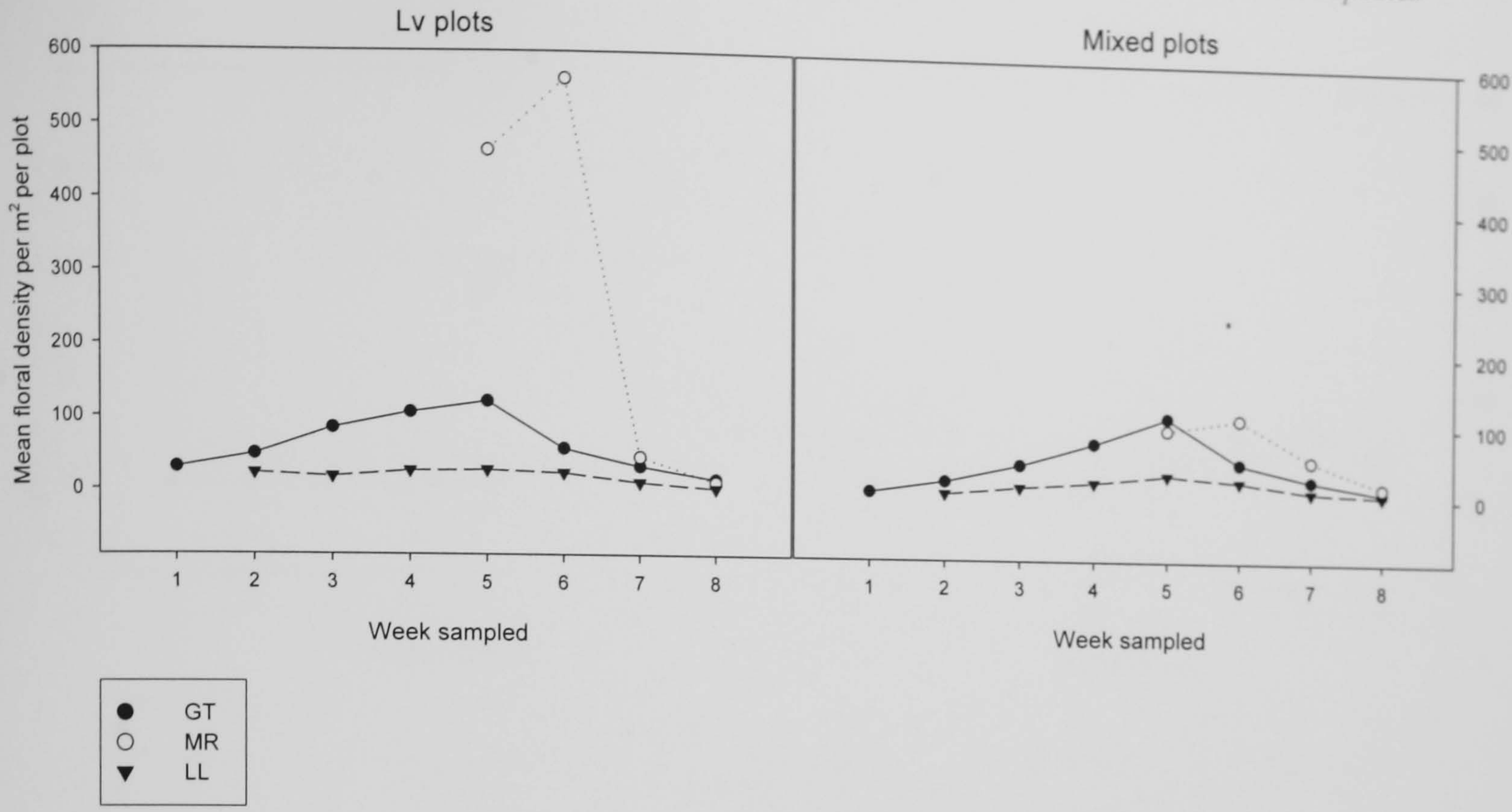


Figure 5.3 Mean floral density per m^2 per plot of *L. vulgaris* at Lv and Mixed plots for all sites sampled over the season. Sampling occurred each week over eight weeks, from mid July (sampling week 1) to mid September (sampling week eight).

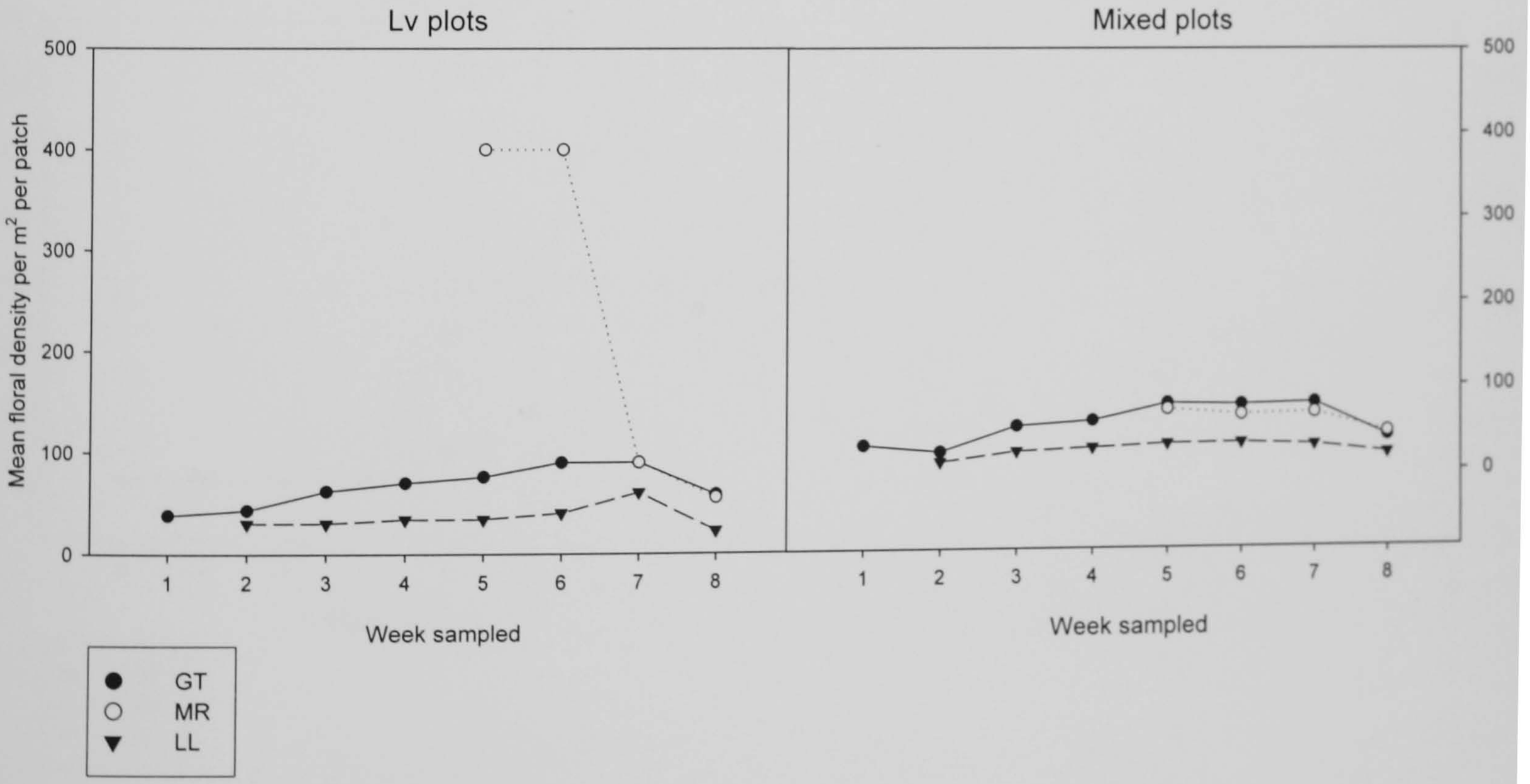


Figure 5.4 Mean floral density per m^2 per patch of *L. vulgaris* at Lv and Mixed plots for all sites sampled over the season. Sampling occurred each week over eight weeks, from mid July (sampling week 1) to mid September (sampling week eight).

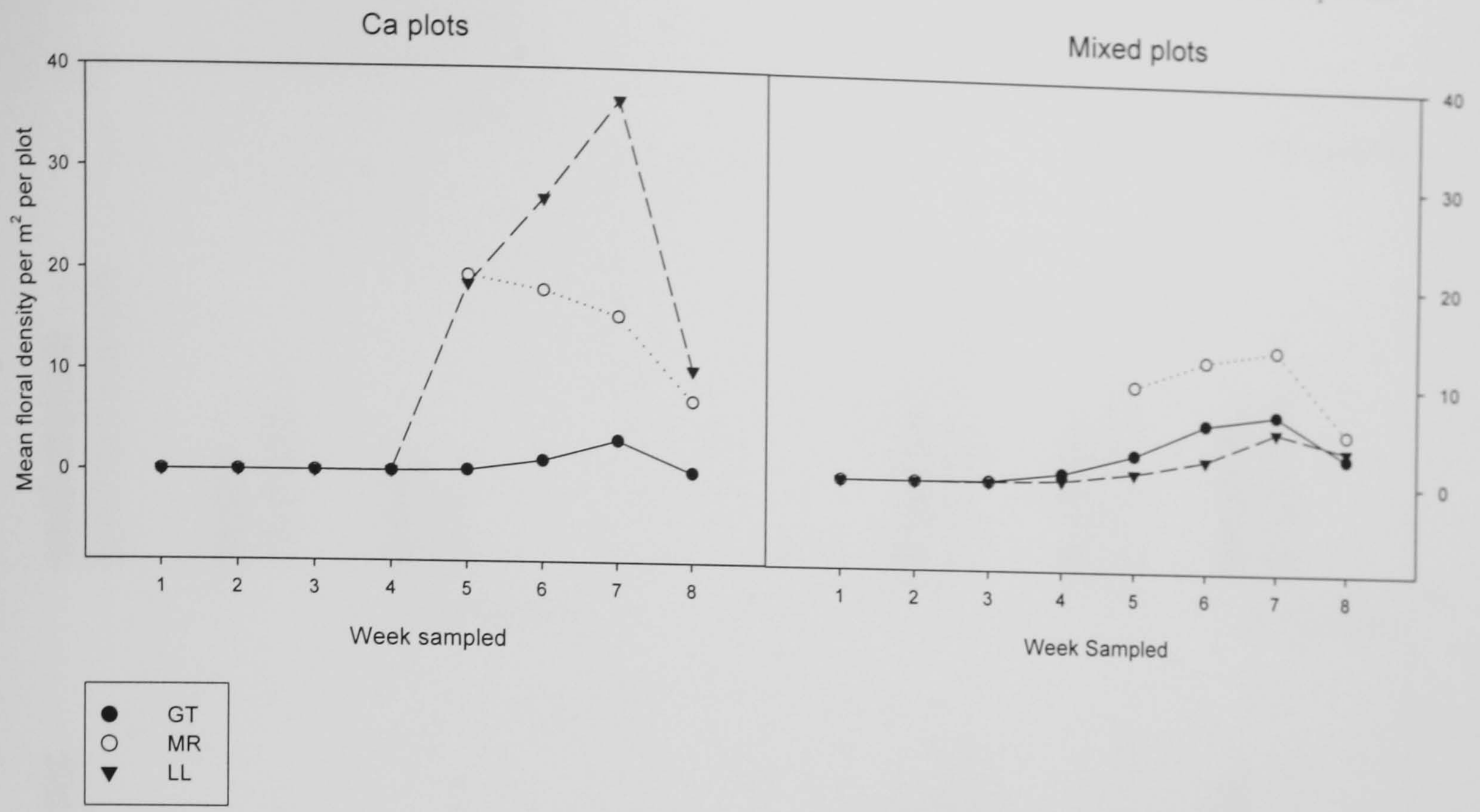


Figure 5.5 Mean floral density per m2 per plot of *C. arvense* at Ca and Mixed plots for all sites sampled over the season. Sampling occurred each week over eight weeks, from mid July (sampling week 1) to mid September (sampling week eight).

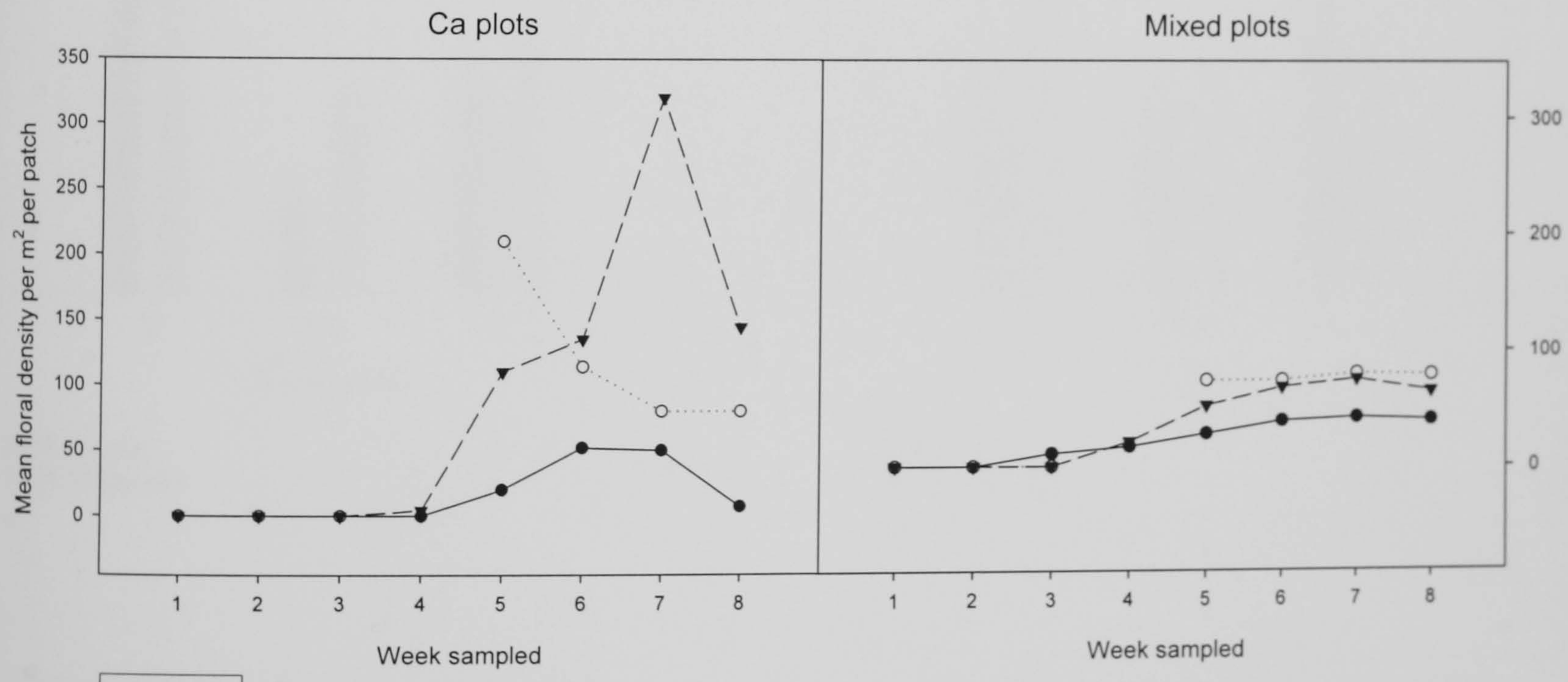


Figure 5.6 Mean floral density per m^2 per patch of *C. arvense* at Lv and Mixed plots for all sites sampled over the season. Sampling occurred each week over eight weeks, from mid July (sampling week 1) to mid September (sampling week eight).

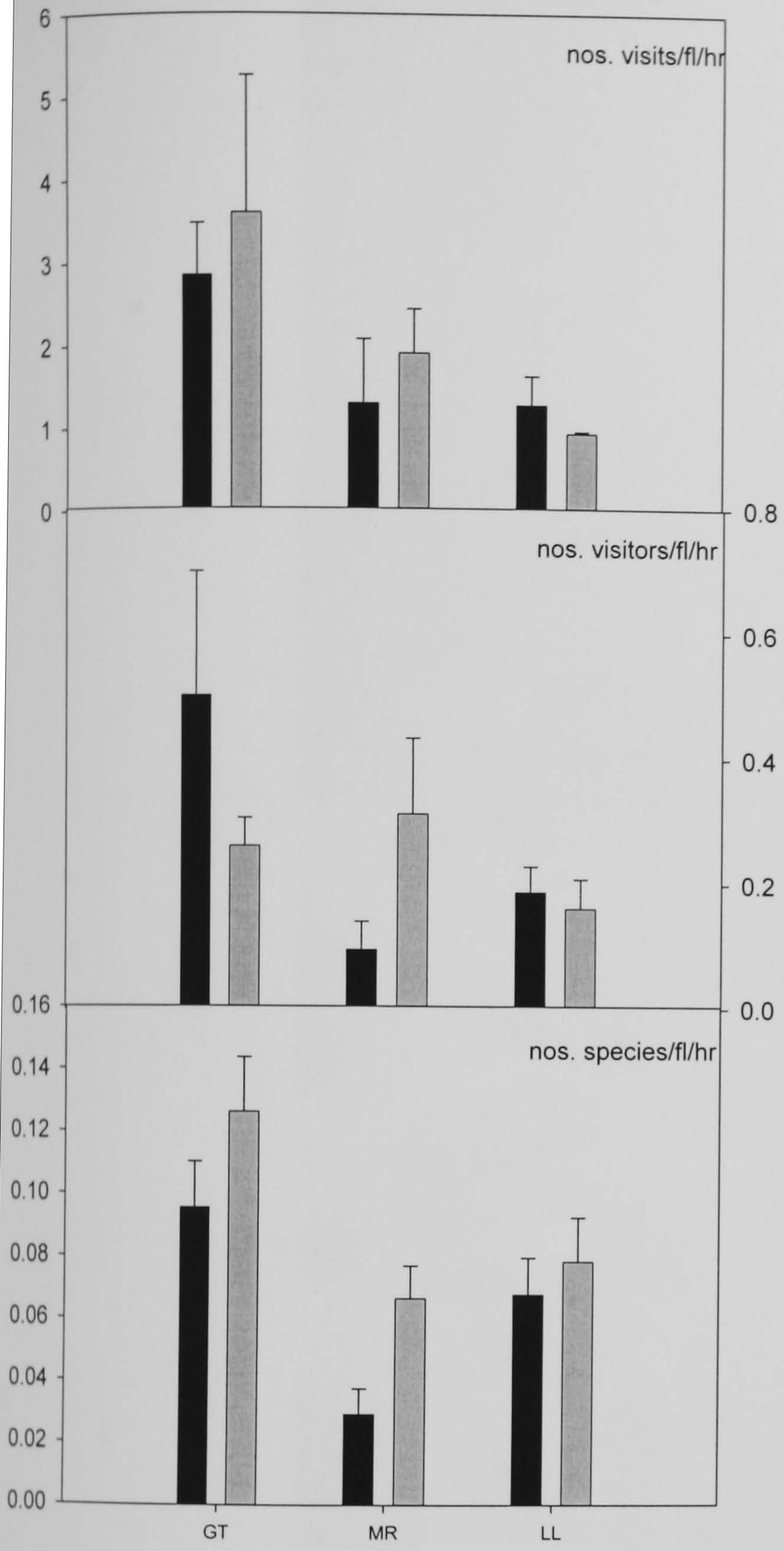


Figure 5.7 Number of visits, visitors, and visiting species per floral unit per hour (± s.e.) to *L. vulgaris* at Lv and Mixed plots for all sites sampled over the season.

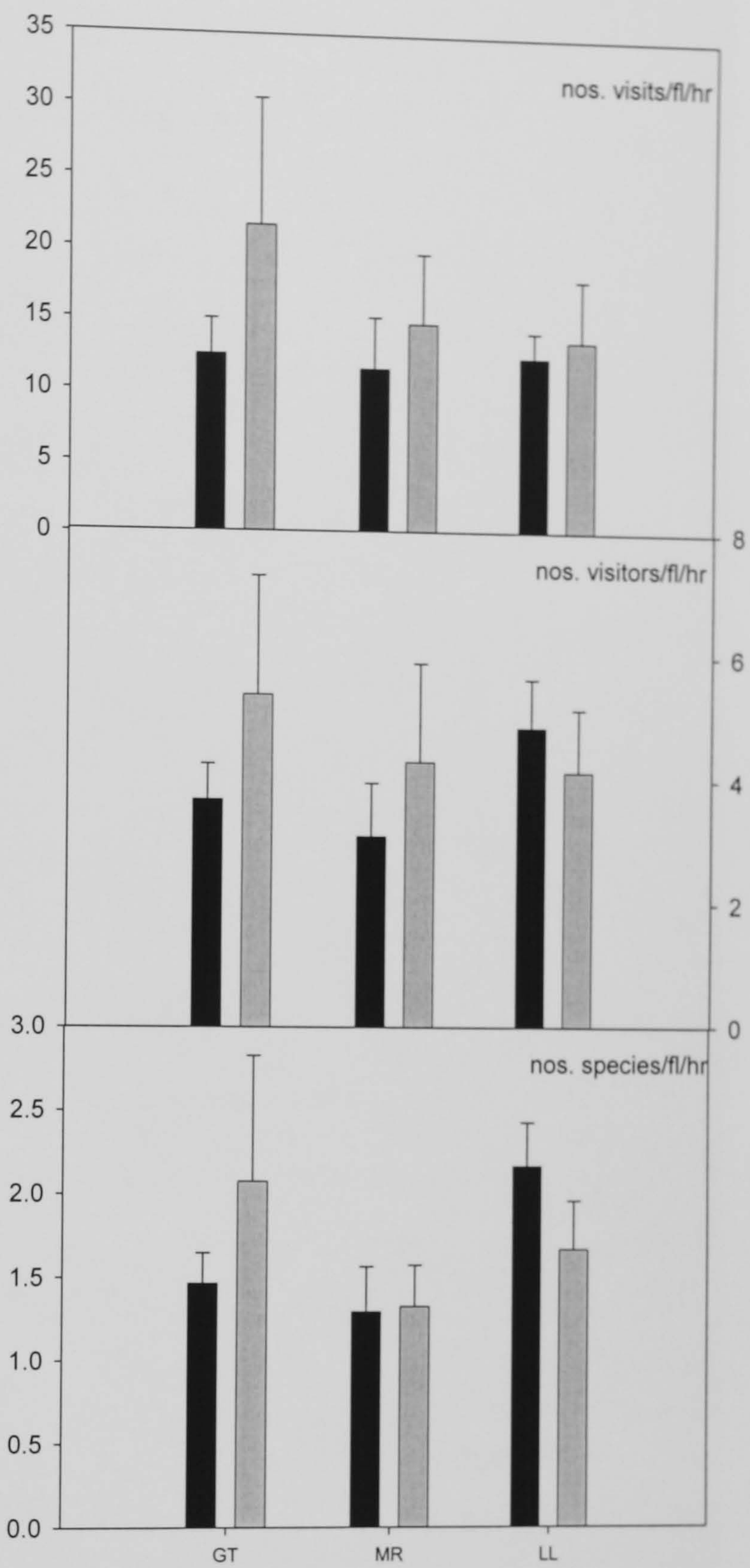


Figure 5.8 Number of visits, visitors, and visiting species per floral unit per hour (± s.e.) to *C. arvense* at Lv and Mixed plots for all sites sampled over the season.

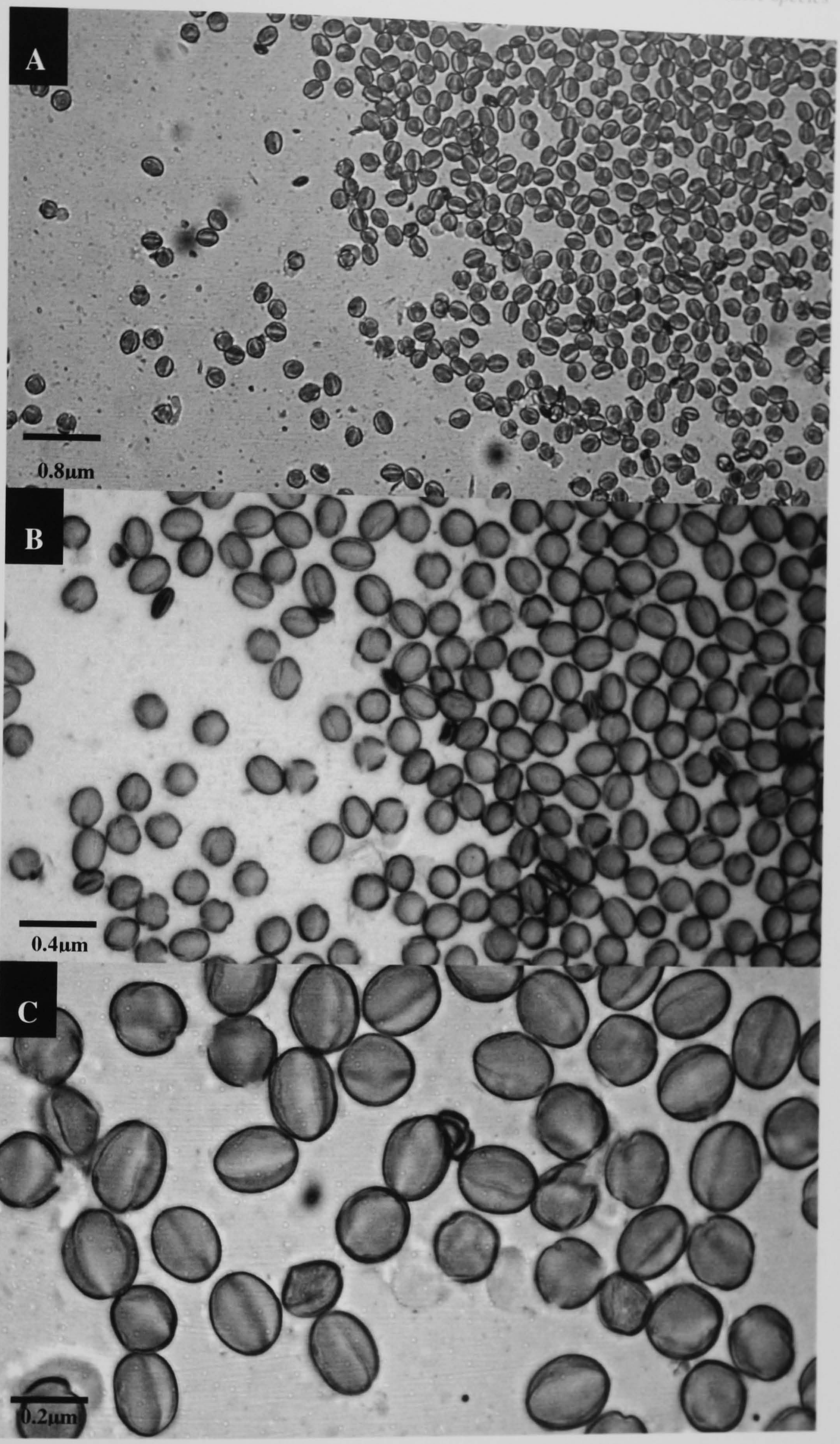


Figure 5.9 Pollen grains of *Linaria vulgaris* (common toadflax, Scrophulariaceae) at x100 (A), x200 (B) and x400 (C) magnification under a light microscope.

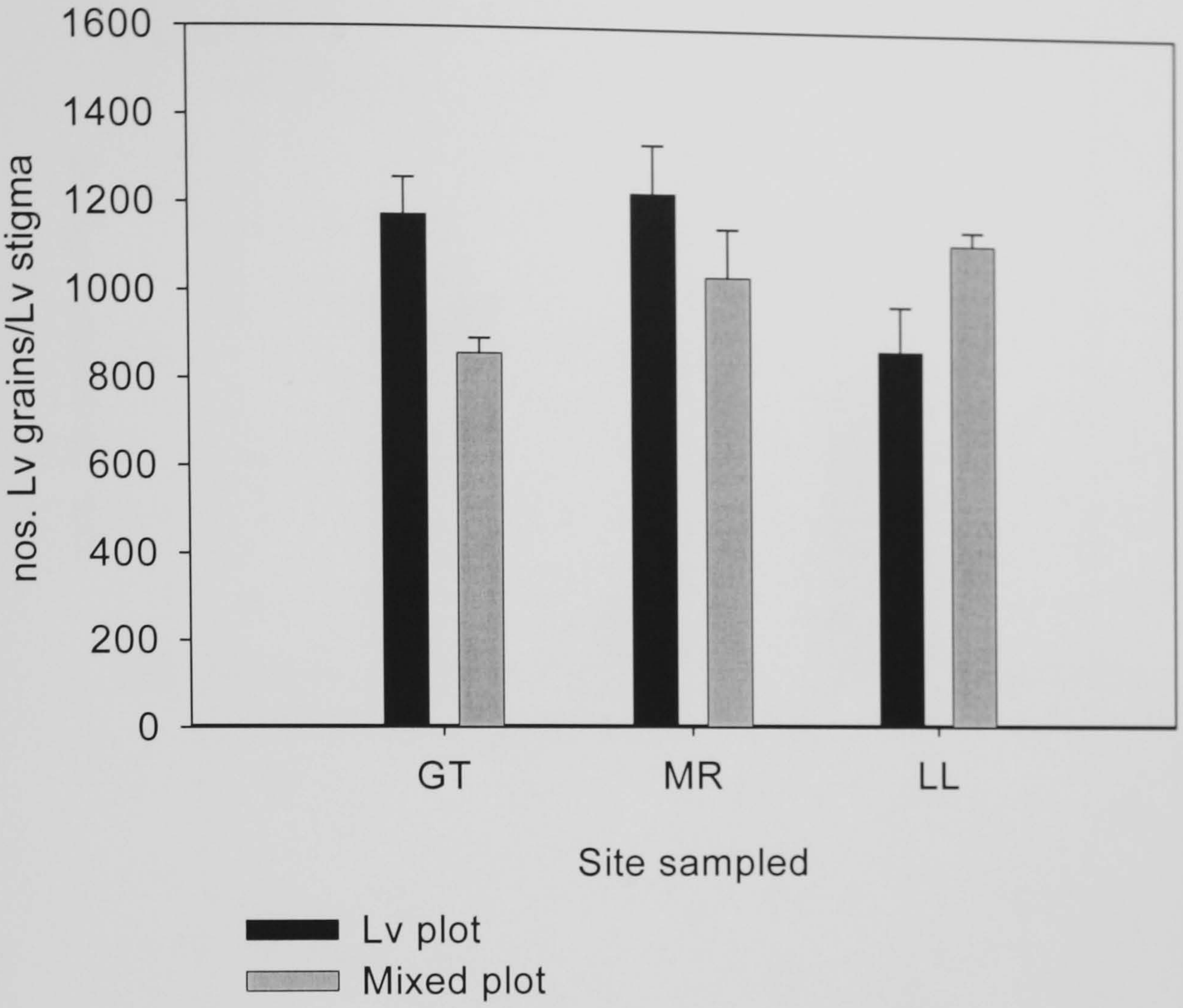


Figure 5.10 Mean conspecific pollen load (± s.e.) found per *L. vulgaris* (Lv) stigma per plot treatment for all sites sampled over the season.

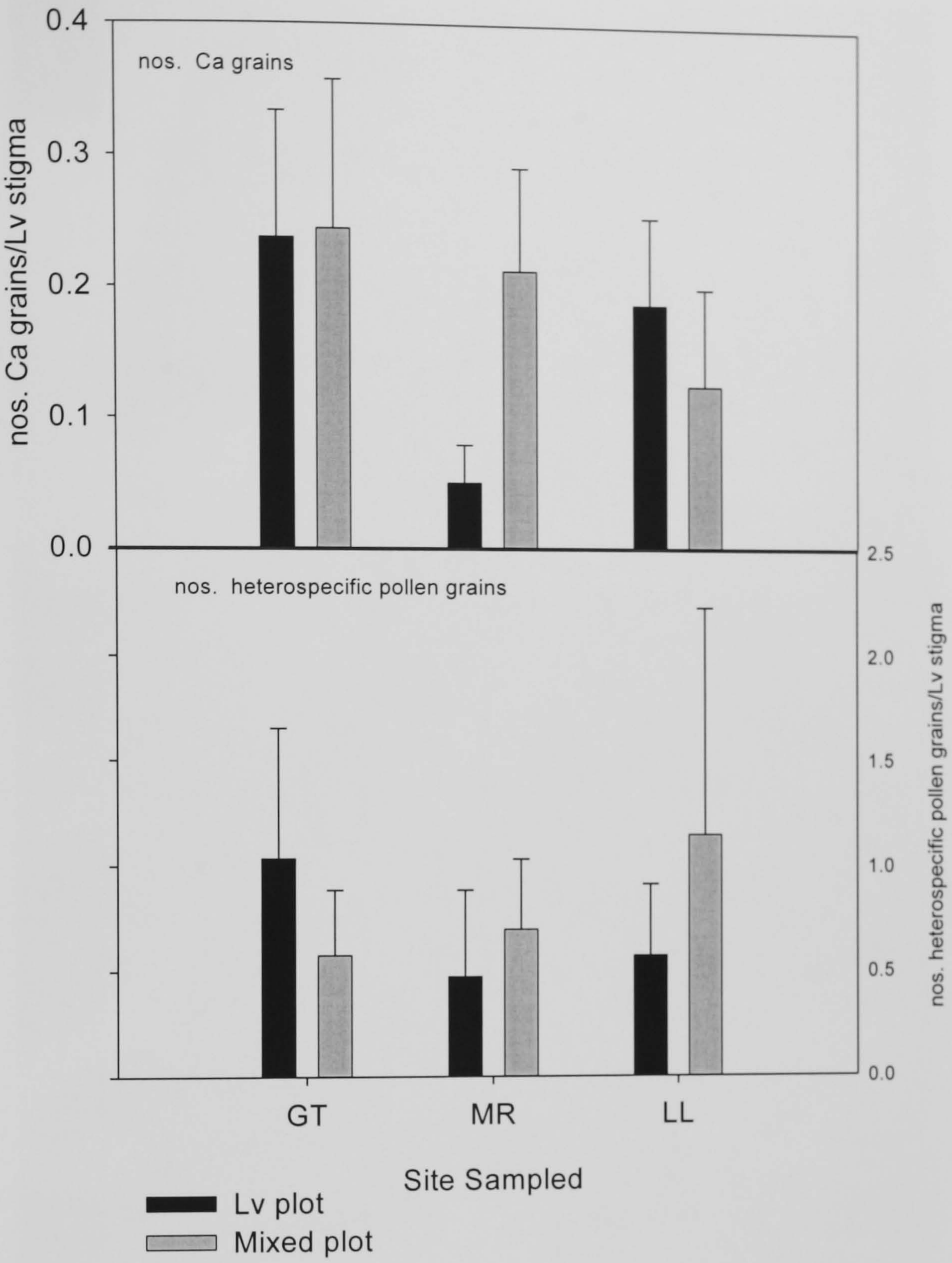


Figure 5.11 Mean heterospecific pollen load (± s.e.) (*C. arvense* (Ca) and other species) found per *L. vulgaris* stigma per plot treatment for all sites sampled over the season.

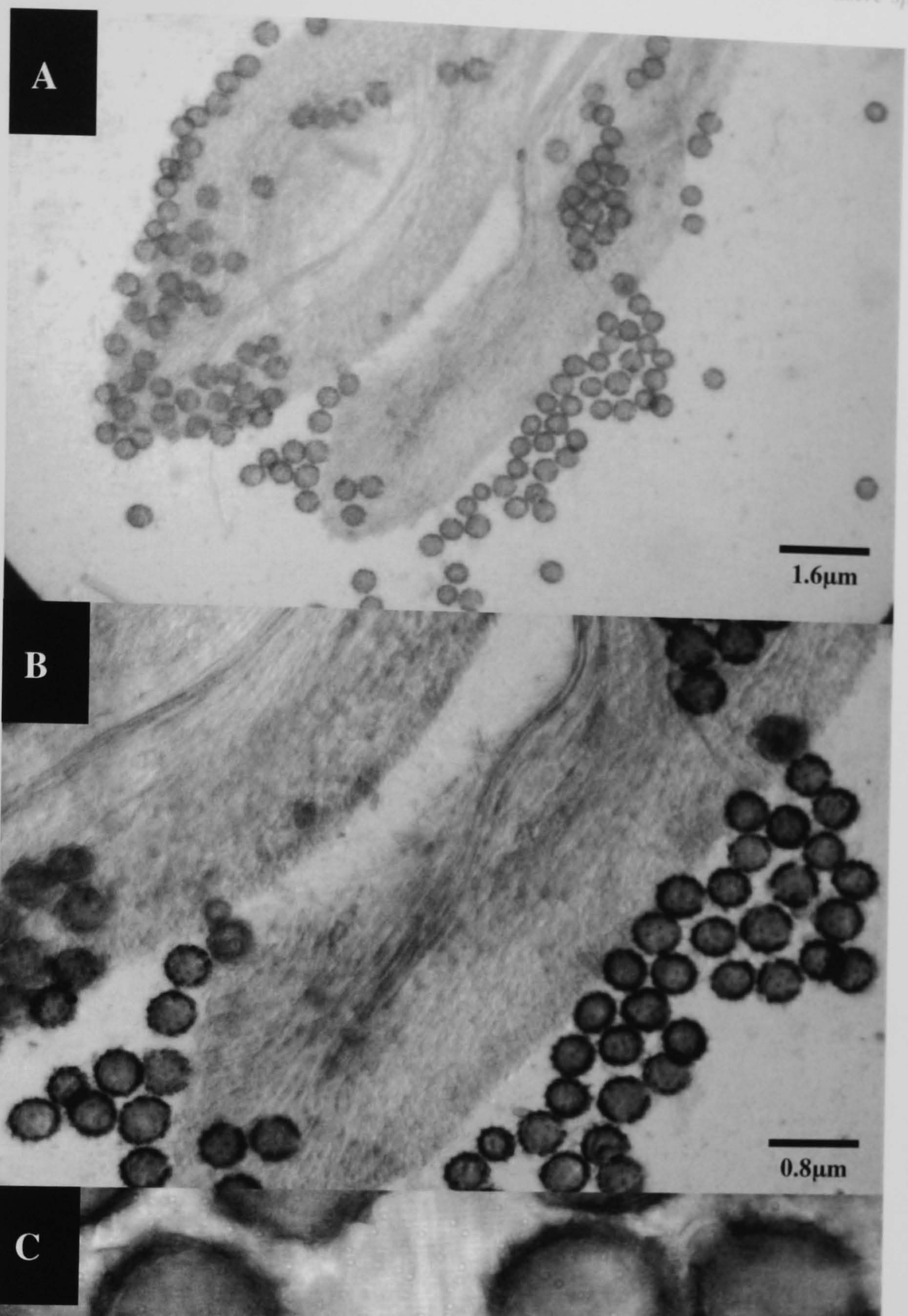


Figure 5.12 Pollen grains of *Circium arvense* (creeping thistle. Asteraceae), as seen on a *C. arvense* stigma, at x50 (A), x100 (B), and x400 (C) magnification under the light microscope.

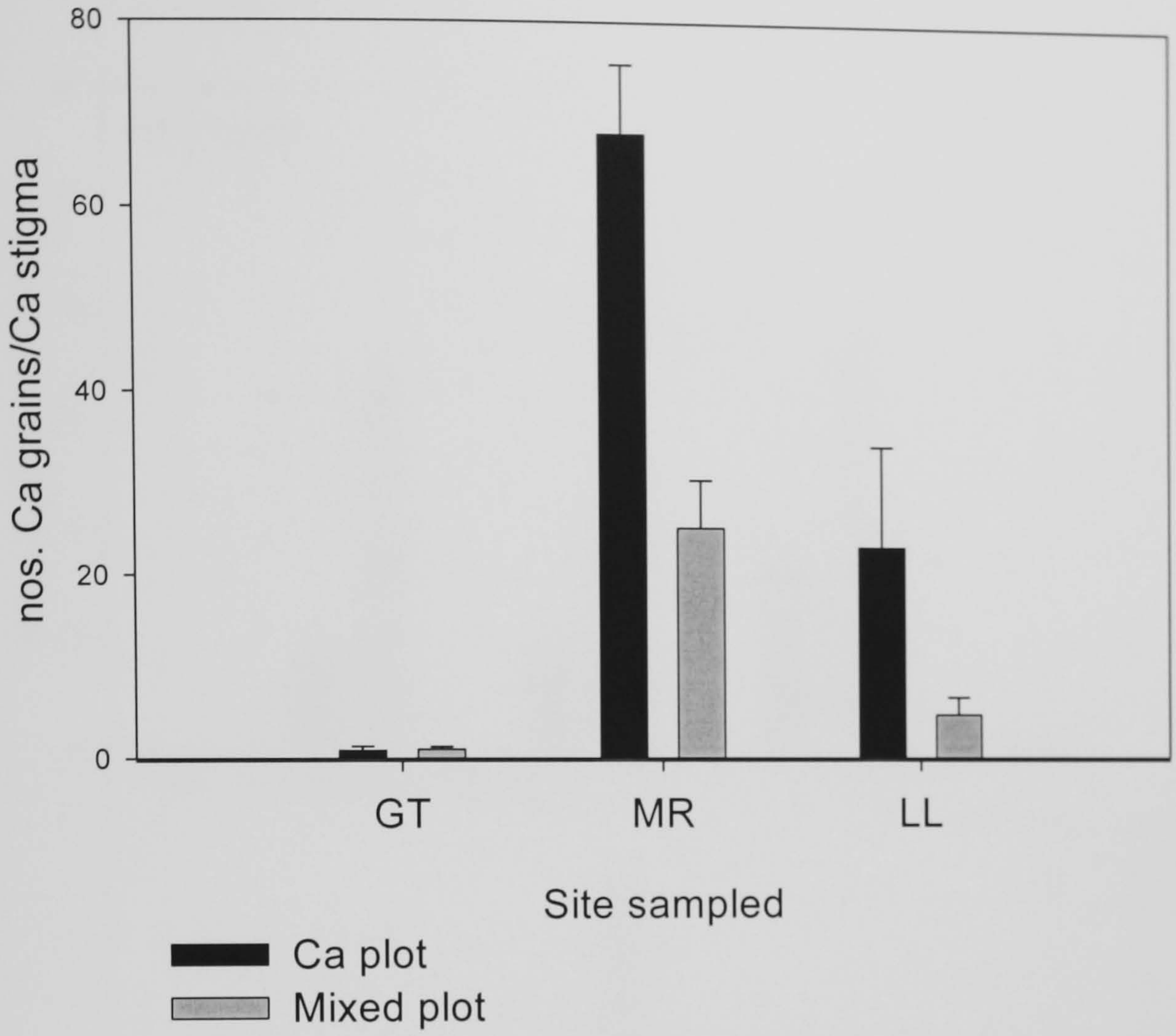


Figure 5.13 Mean conspecific pollen load (± s.e.) found per *C. arvense* (Ca) stigma per plot treatment for all sites sampled over the season.

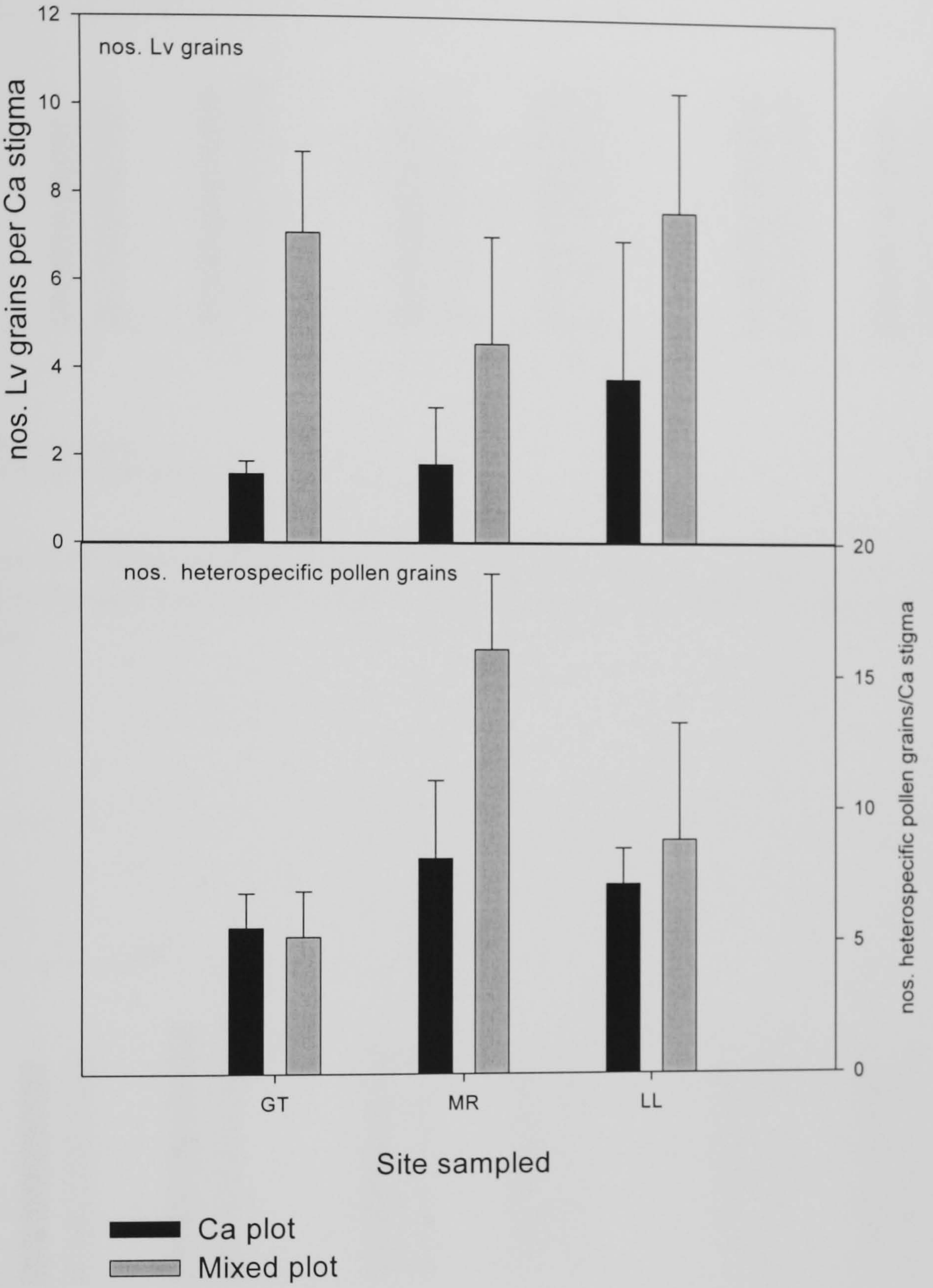


Figure 5.14 Mean heterospecific pollen load (± s.e.) (*L. vulgaris* and other species) found per *L. vulgaris* stigma per plot treatment for all sites sampled over the season.

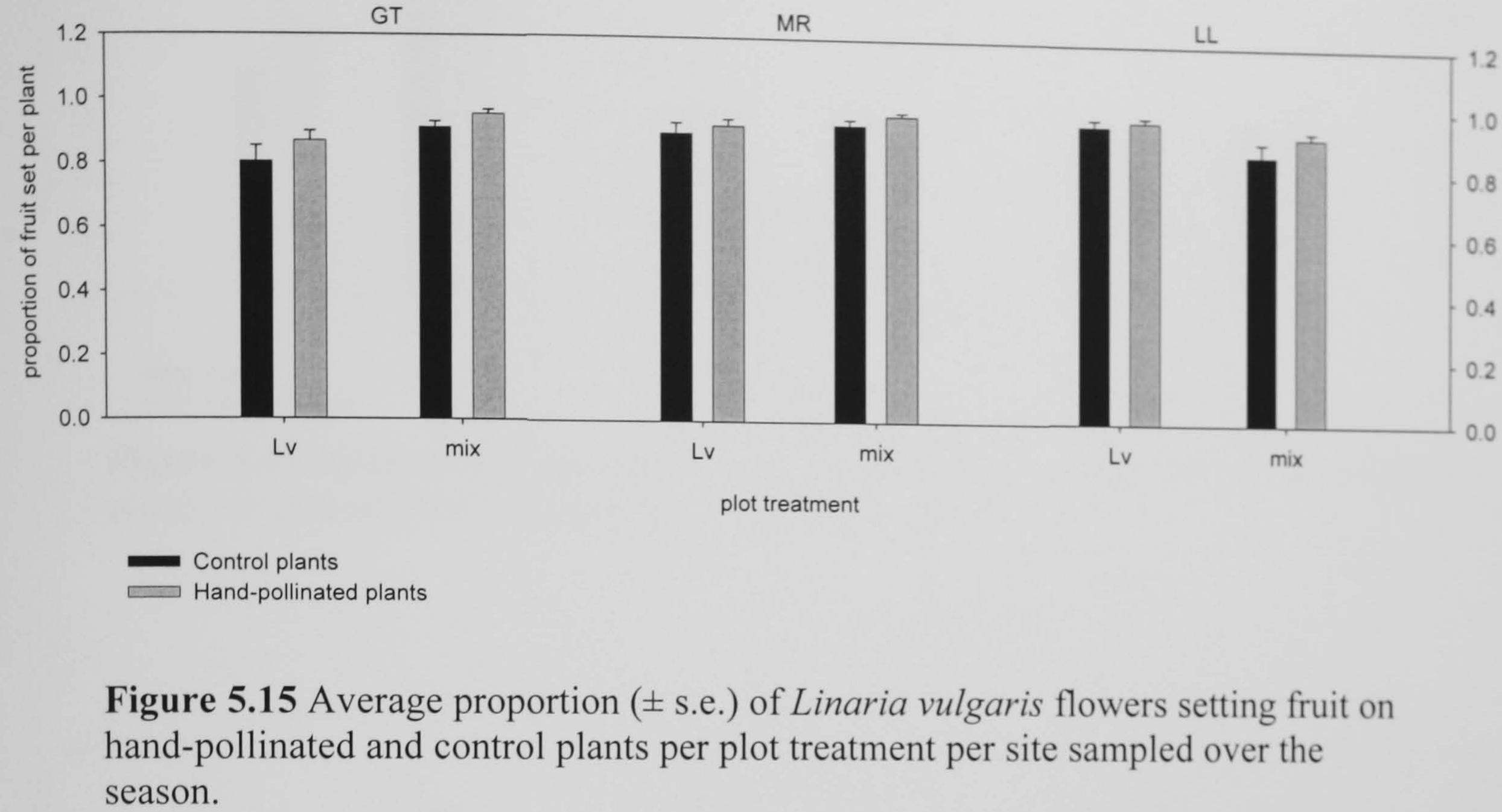


Figure 5.15 Average proportion (± s.e.) of *Linaria vulgaris* flowers setting fruit on hand-pollinated and control plants per plot treatment per site sampled over the season.

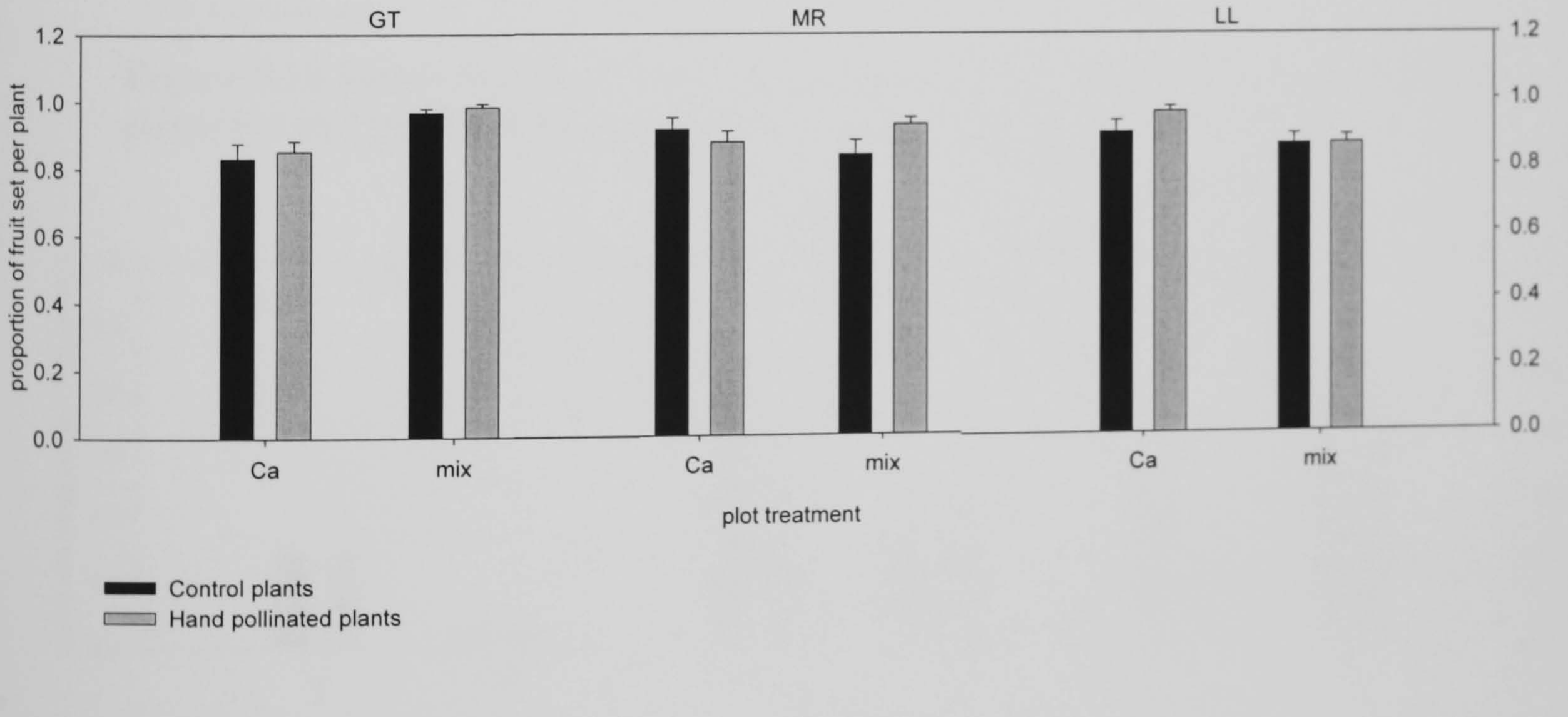


Figure 5.16 Average proportion (± s.e.) of *Cirsium arvense* flowers setting fruit on hand-pollinated and control plants per plot treatment per site sampled over the season.

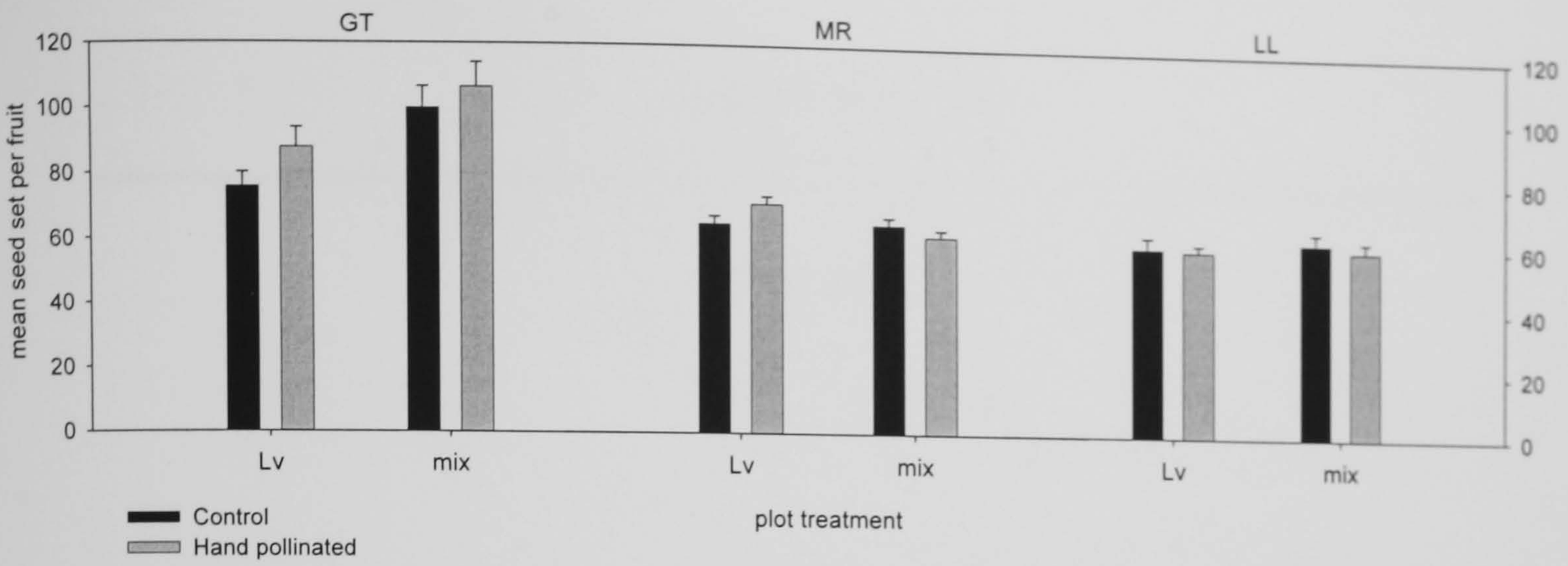


Figure 5.17 Mean seed set (± s.e.) for *L. vulgaris* fruit of hand-pollinated and control plants per plot treatment per site sampled over the season.

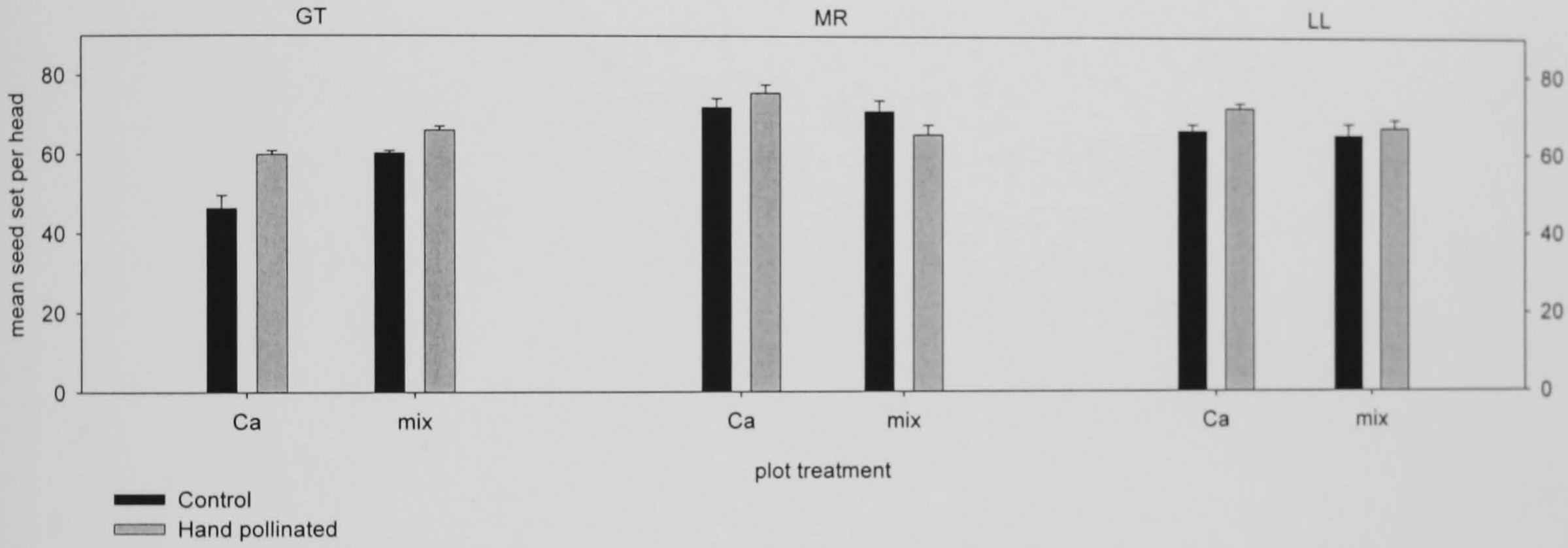


Figure 5.18 Mean seed set (± s.e.) for *C. arvense* fruit of hand-pollinated and control plants per plot treatment per site sampled over the season.

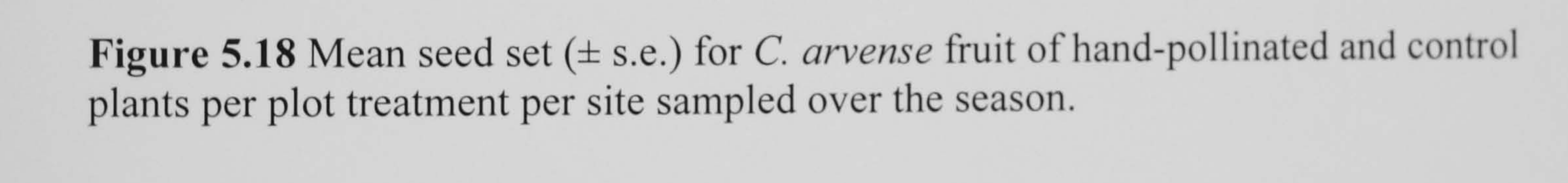


Figure 5.19 Mean seed set (± s.e.) for *C. arvense* fruit of hand-pollinated and control plants per plot treatment per site sampled over the season.

CHAPTER SIX

Discussion

6.1 Discussion

The studies described in this thesis primarily evaluate the potential for ecological restoration to reinstate essential ecological interactions to previously degraded communities. In Chapter 2, I assessed the restoration of plant-pollinator interactions to heathland communities following eleven and fourteen years of restorative management, and in Chapter 3, a third trophic level was further investigated. Here, the interactions between the heathland flora, their bumblebee pollinators, and the natural enemy community of these pollinators were studied to determine the potential for the restoration of higher trophic levels to communities. Chapters 4 and 5 then went on to consider the extent and impact of specific ecological interactions that are potentially implicated in the restoration process. Chapter 4 investigated further the patterns and prevalence of the "hidden" community of bumblebee natural enemies, providing important baseline data on bumblebee parasitism, whilst Chapter 5 questioned the potential for facilitatory interactions between alien invasive species, with regard to evaluating the processes needed to be overcome for the management of their invasive spread. In this final chapter I synthesise the main findings of this thesis. I then go on to consider the conservation and management implications of this work in the wider context of restoration ecology, before outlining the potential future directions of research.

What has been learnt?

Ecological restoration requires both the structural and functional attributes of ecosystems to be in place before a self-sustaining, self-regulating community is successfully reinstated. Restoration projects in the past have typically focussed on restoring ecosystem structure, using readily measurable attributes such as species richness and abundance to confirm successful reinstatement to restored sites. Yet by solely studying the structural aspects of a community, we remain uninformed about what species actually do. However reinstating ecosystem function to restored sites and deciding upon its measurable attributes is considerably harder, and further constrained by both the duration and cost of restoration projects.

As outlined by the Society for Ecological Restoration (SER 2004), restored communities should be well integrated with the landscape, resilient to natural disturbances, and show evidence of self-sustainability and persistence through environmental variation. Yet how can we even begin to evaluate such broad functional attributes, particularly along an ever-changing restoration trajectory? Identifying the foundations upon which ecosystem function lie provide a good starting point. Such a foundation is created by the interactions that occur between species. These interactions are essentially the drivers of ecological processes, processes which are responsible for community and ecosystem functioning that underlie the provisioning of ecological goods and services. Thus by considering species interactions in evaluating restoration success, we are effectively working in the currency of ecosystem services (Memmott *et al. in press*).

By elucidating key interactions within and between trophic levels of a community, important functional subsets of the network can be quantified, enabling a truer evaluation of both structural and functional parameters at restored relative to reference or target communities (e.g. Forup *et al. in press*). Such functional subsets studied throughout this thesis included the plant-pollinator and pollinator-parasite interaction networks of ancient (reference) and restored dry, lowland heathland communities.

The importance of measuring both structural and functional parameters concurrently when evaluating restoration success was highlighted in Chapter 2, when gauging the success of the reinstatement of a pollination service to communities undergoing ecological restoration. Here, although the structural components of the plant-pollinator community (such as species abundance, diversity and composition) were successfully reinstated at restored communities, functional differences remained, with the density and complexity of species interactions reduced at restored relative to reference ancient communities, despite fourteen years of restorative management. However the magnitude of this difference was small relative to other comparative studies. Indeed the positive relationship found between the density of species interactions (i.e. the 'connectance' of a community network) and the robustness of those communities to species extinctions documented by Dunne *et al.* (2002) involved a comparative analysis of communities whose connectance differed by tenfold, compared to the restored and ancient community networks documented in this study where differences in connectance were less than twofold. Thus only by

being informed by both structural and functional measures could restoration success of the plant-pollinator community be effectively evaluated, concluding here that that both the structural and functional components of the plant-pollinator community were sufficiently reinstated. This is particularly important as considerable alterations in species interactions can have little impact upon standard measures of community structure such as species diversity or richness (Tylianakis *et al.* 2007).

Restoration ecology has the potential to both inform and be informed by ecological theory. Yet the integration of research between trophic ecology and restoration ecology remains rare (Young *et al.* 2005). In Chapter 3, the assessment of species interactions was expanded to studying three trophic levels, incorporating the natural enemy community of bumblebee pollinators into the networks. Here I show that such higher-ranked trophic specialists can be useful indicators in the evaluation of restoration success of previously degraded communities. Healthy ecosystems are those that are typically parasite-rich (Marcogliese 2005; Dobson *et al.* 2006), with the loss of parasite species in degraded systems altering host abundance and dominance. This can shift interspecific competition to the detriment of the overall biodiversity. In this study, the restoration of both the structural and functional components of the community network remained incomplete, with the establishment of a key dipteran parasitoid significantly reduced at restored relative to reference sites despite essential resources being in place. Drawing from community assembly theory, such higher-ranked trophic specialists are typically more susceptible to resource fluctuations due to their small population size and their reliance upon the presence and abundance of their host and their host's resources. Consequently parasites have been used successfully as bioindicators when assessing communities for environmental stress, food web structure, and biodiversity (reviewed by Marcogliese 2005). However the study documented here remains one of only a few that use parasites in evaluating restoration success, whilst further highlighting the usefulness of a food web approach to ecological restoration - a tool that has only recently gained recognition in restoration ecology (Zanden *et al.* 2006; Forup *et al. in press*).

Over the past decade, pleas calling for the inclusion of parasites into community studies have received limited responses. Yet recently it has been shown that upon the inclusion of parasites to food web studies, key descriptors can be altered, and sometimes dramatically. For example, increases in species richness,

linkage density and network connectance were documented upon the addition of parasite information to key food-web studies (Lafferty *et al.* 2006). Thus the incorporation of such "hidden players" in networks studies are important, particularly as such web descriptors infer important attributes such as robustness of the community to species extinctions and environmental perturbations (Ives & Cardinale 2004; Dunne *et al.* 2002). Indeed parasites are known to be important regulators of community structure and function (Dobson & Hudson `986; Minchella & Scott 1991), mediating a range of competitive and predatory interactions and stabilising host populations (Price *et al.* 1986; Prenter *et al.* 2004). Furthermore, their ubiquitous nature alone accounts for a large proportion of the biodiversity itself (Dobson *et al.* 2006). Yet their omission from community studies leaves them poorly understood. Thus although they have been shown here and elsewhere to be potentially good indicators of restoration success (Huspeni & Lafferty 2004), the difficulties in obtaining adequate baseline data on reference parasite communities limits their application across the wider diversity of restored communities and habitats. For example, quantifying the bumblebee-parasite interactions at ancient sites was essential to provide reference data against which the interactions at restored sites could be compared. Furthermore, it was necessary to replicate such baseline data over several reference and restored sites to determine their spatial variation. Yet such an intensive study is likely unfeasible for the vast majority of restoration projects, given their economic and time constraints. Readily available baseline data are thus a potentially valuable resource to restoration ecologists.

Furthermore, research on the community of bumblebee parasites present in the UK remained, up until now, in the hands of Alford, who offered the last comprehensive, qualitative assessment of the natural enemy community of bumblebees back in 1975. Yet studies documenting bumblebee parasite prevalence and extent are key to furthering our understanding of the impact of parasitism in light of bumblebee decline, as well as investigating the otherwise hidden and oft ignored biodiversity of these natural enemies. I therefore further examined the patterns and prevalence of parasitism in bumblebee communities, thereby providing the first quantitative assessment of parasitism of bumblebee foragers of dry lowland heathland in England.

Such data are also important when considering the increasing global importation and exportation of bumblebee colonies for pollinator services over the

last ten years. Natural enemies are easily displaced following the importation or accidental introductions of their bumblebee hosts. Past studies have described the infection of native bumblebee hosts with novel pathogens introduced together with imported colonies into Japan (Goka *et al.* 2006), USA, Mexico and Canada (Winter *et al.* 2006), whilst in New Zealand, introduced bumblebee populations have undergone rapid expansions probably as a consequence of enemy release. Moreover an estimated 10,000 colonies of the non-native subspecies *Bombus terrestris dalmatinus* are imported annually into the UK for pollination services (Ings *et al.* 2005). This subspecies has been considered a potential threat to native conspecifics, due to their higher reproductive success, superior foraging ability, and larger colony size (Ings *et al.* 2006). Baseline data that quantify the patterns and prevalence of parasitism for native bumblebee communities are therefore also important when determining the impacts of introduced species. For example, such data can inform management protocols of introduced species by determining the potential for native enemies to mediate competitive interactions between introduced subspecies and native conspecifics.

Understanding and identifying key interactions that have the potential to impact upon native communities are essential if ecological restoration and conservation management of our natural resources are to be successful. With ecological restoration armed with the task of slowing down and ultimately reversing habitat loss, it is simultaneously faced with the second biggest threat to biodiversity: the establishment and subsequent spread of non-native species. Indeed ecological restoration of degraded habitats typically involves the removal and/or subsequent prevention of invasive species spread when restoring degraded communities. Identifying interactions that drive biological invasions is therefore crucial when undertaking restoration management.

Theoretical models that predict species invasiveness or the invasibility of a community however, are typically limited as a consequence of their predominant focus on negative interactions (Boucher *et al.* 1982; Bertness & Callaway 1994) assuming no potential for facilitation between invasive species. Yet with the exponential rise in the global spread of alien species, the potential for positive interactions, whether direct or indirect, is increasingly likely, particularly upon the reunion of mutualistic partners in their invaded range (Parker *et al.* 1999; Richardson *et al.* 2000; Grosholz 2005). In Chapter 5 I attempted to determine the potential for

positive interactions between two invasive plant species, with regard to the facilitation of their reproductive success and subsequent spread within the native community. Although no positive interactions were found to occur in this study, it remains important to consider the potential for facilitation between non-native species for any community. Evidence for facilitation between non-native invasive species, although scarce, has been documented for coastal, freshwater, and terrestrial ecosystems. Yet such positive interactions between introduced species can have huge implications for the native community. O'Dowd *et al.* (2003) documents an example of this for Christmas Island. In this study, the invasive yellow crazy ant caused the death of the native red land crab, the dominant consumer on the forest floor. Seedling recruitment was consequently released, enhancing seedling species richness and slowing litter breakdown. The invasive ant also interacted with a previously introduced homopteran species, tending its honeydew and thereby sustaining high population densities of this homopteran in the canopy. The deposition of excess honeydew on canopy leaves encouraged the growth of sooty moulds that resulted in the dieback of the canopy and death of canopy trees. Here, the potential for positive interactions between multiple non-native species to have synergistic effects upon the native community is realised: the total impact of the invader complex far exceeding the sum of individual impacts, precipitating an "invasional meltdown". Thus the potential for positive interactions to factor in determining the extent of an invasion should be considered at the early stages of invasive spread, as the cost of mitigating future impacts arising from such synergistic interactions between invader complexes will undoubtedly outweigh the cost of their current control and prevention.

Conservation and management implications

> *"That most insidious type of extinction, the extinction of ecological interactions"*
>
> (Janzen 1974)

The increasing pressures of an expanding human population together with the impacts of global climate change will exacerbate current rates of habitat loss and biological invasions without further intervention. Yet conservation management of endangered and threatened habitats and species alone is no longer viable, given the

acceleration of fragmentation and isolation of such habitats eroding the viability of their species populations. The need for ecological restoration to reinstate communities to previously degraded habitat is therefore urgent if current levels of biodiversity and ecosystem functioning are to be maintained or increased.

As outlined in Chapter 1, the resolution of both conservation and restoration management in the past has focused at the level of species. Yet as emphasised throughout this thesis, a far more informative approach can be achieved through the study of species interactions; their importance first recognised thirty years ago by Janzen (1974) as stated in his quote at the start of this section. Together with the appropriate analytical tools such as the application of food web theory to community studies, a more holistic approach to determining key community attributes can be undertaken. Such attributes include those that describe community stability and robustness to environmental perturbations and species extinctions, as described in Chapters 1 and 2. Furthermore, the use of such descriptors for both target reference and restored communities can allow for an effective, comparative evaluation of restoration success, by determining the restoration of both community structure and function simultaneously (Chapters 2 & 3).

Other implications for conservation management have also arisen throughout the studies undertaken in this thesis. Firstly, it was found that heathland sites chosen for restoration need not stand adjacent to reference heathlands for the successful reinstatement of their insect community, whilst emphasising the importance for both temporal and spatial replication to be incorporated into ecological restoration programmes (Chapter 2).

Secondly, the natural enemy community of bumblebees can act as good indicators of restoration success, and in the case of heathland habitat, could provide a useful yardstick with which to measure the reinstatement of higher trophic interactions as a surrogate for the restoration of community function (Chapter 3). Yet despite many studies documenting parasites as potential bioindicators for ecosystem health and biodiversity, only three to my knowledge incorporate such 'hidden players' in the evaluation of restoration success: Cone *et al.* (1993); Valtonen *et al.* (2003) and Huspeni & Lafferty (2004).

Thirdly, the provision of baseline data regarding the prevalence and patterns of bumblebee parasitism (Chapters 3 & 4) may not only provide a useful comparison for other restored communities, but may also inform conservationists with regard to

the current 'health' of bumblebee populations, particularly in light of their current decline. Furthermore, despite taking a more holistic view in studying species interactions, information on the abundance and diversity of the individual species of the natural enemy community of bumblebees can also be gleaned. For example, the key dipteran parasitoid family, Conopidae, are themselves endangered: of the 24 species recorded for the UK, only 6 species are considered common, with 2 recorded in the Red Data Book as endangered, 11 as nationally rare, and 5 as scarce/nationally notable. Thus further declines in their host species render these parasitoids further susceptible to extinction.

Future directions

This thesis reveals the potential for ecological restoration to provide an arena for the development and testing of ecological theory. With most fundamental ecological theory based on natural systems generally free of anthropogenic pressures, restoration ecology, which deals with the recovery of degraded systems following damage, is a valuable informant. Conversely, restoration ecology is frequently guided by ecological theory itself, incorporating fundamental principles such as community succession, community assembly rules, and population dynamics, into conceptual restoration models.

Yet despite Bradshaw emphasising the potential for restoration to provide the ultimate "acid test" of our understanding with regard to the functioning of ecosystems (Bradshaw 1987), the evaluation of ecosystem function remains poorly incorporated into restoration programmes. Indeed really only in the past decade have theoretical and empirical research regarding the link between ecological structure and function really grown within the field of ecology itself, including research relating biodiversity and ecosystem function (e.g. Naeem *et al.* 1994; McGrady-Steed *et al.* 1997; Ives *et al.* 2000), functional redundancy of species (Lawton & Brown 1993; Naeem 1998), and the study of network properties that govern a community's resilience to extinctions and environmental perturbations (Dunne *et al.* 2002; Ives & Cardinale 2004; Memmott *et al.* 2004).

Thus only now is restoration ecology beginning to recognize the importance of understanding such relationships between the structural and functional attributes

of communities to be restored, moving away from a focus solely on the former to one that incorporates the latter. The work described throughout this thesis in particular, highlight five main areas in need of further research:

1) Ecological restoration must begin to incorporate a food-web perspective by including species interactions in community restoration goals. Such interactions can be effective surrogate measures of functional attributes when evaluating restored systems. For example, by assessing the reinstatement of key interactions from which important functional processes arise (such as seed dispersal, pollination, and biological control), the extent to which a restored community is self-sustaining and self-regulating can be determined.

2) The use of interaction networks and food web theory as a predictive tool in determining restoration outcomes should begin to be explored. Current theoretical advances in analysing such networks have gone so far as to determine key properties that govern a community's resilience to species extinctions and environmental perturbations. Such a tool could also prove invaluable for restoration in the future, such as by determining thresholds above which restored communities can be deemed sustainable, or by predicting potential impacts of alien species removal at different points along a restoration trajectory.

3) The use of higher trophic level specialists such as parasites and parasitoids should be used more frequently when evaluating community restoration, as their presence is indicative not only of the availability of their hosts, but also of their hosts' requirements.

4) Further research into the impact of bumblebee parasitism on both declining and imported species should be urgently pursued. With evidence documenting resource-stressed bumblebee hosts more susceptible to parasitism, and with the loss of resources identified as a key factor in the continual decline in threatened bumblebee species, a comparative analysis of the prevalence and susceptibility to parasitism between declining and ubiquitous species may shed further insight into understanding their decline. Also, with the continual annual importation of non-native subspecies of bumblebee, further research into understanding the potential for parasitism to mediate competition between non-native and native conspecifics should be undertaken, particularly in light of evidence for the superiority of non natives with regard to foraging and reproductive success (Ings *et al.* 2006).

5) The potential for facilitative interactions between invasive species is an essential avenue of research, particularly with the exponential rise in their global spread increasing the likelihood for the build up of invader complexes. Furthermore, with increasing evidence documenting synergistic interactions involving both recent and historical invasions, a more prudent approach to their management is needed. By understanding the extent of such interactions restoration ecologists will not only be better informed with regard to the implementation of effective preventative measures, but will also better able to evaluate the impacts of their removal from the overall community network.

More generally however, the continual decline in global biodiversity and the associated losses of crucial ecosystem services, in spite of numerous conservation policies implemented to protect habitat types and species, highlights the urgency for ecological restoration to take a more dominant role in habitat and ecosystem management. Indeed restoration ecologists are now armed with the necessary analytical tools and are well-informed by ecological theory with regard to community processes and ecosystem functioning. Furthermore, the integration of such theory and the use of more vigorous scientific approaches in the assessment of ecological restoration is a growing direction in restoration ecology. Such positive advances are encouraging; however for ecological restoration to keep apace, restoration ecologists urgently need to: firstly increase the integration of scientific experimental testing into the restoration arena to further advance our understanding of the dynamics of communities and ecosystems; secondly step up their efforts in identifying those key interactions and processes that underlie community and ecosystem functioning from the very outset of restoration; and finally, undertake effective evaluations through the post-monitoring of restoration programmes with regard to determining restoration success in reinstating both structure and function to restored communities throughout the restoration trajectory. Only then will the potential for ecological restoration to reverse current trends in global biodiversity declines be fully recognised.

REFERENCES

REFERENCES

Adams M.J., Pearl C.A. & Bury R.B. (2003) Indirect facilitation of an anuran invasion by non-native fishes. *Ecology Letters*, 6, 343-351

Aizen M.A. & Feinsinger P. (1994) Habitat fragmentation, native insect pollinators, and feral honeybees in Argentine chaco serrano. *Ecological Applications*, 4, 378-392

Alford D.V. (1968) Biology and immature stages of *Syntretus splendidus* (Marshall) (Hymenoptera - Braconidae Euphorinae) a parasite of adult bumblebees. *Transactions of the Royal Entomological Society of London*, 120, 375

Alford D.V. (1975) *Bumblebees.* Davis Poynter, London, U.K

Allen-Wardell G., Bernhardt P., Bitner R., Burquez A., Buchmann S., Cane J., Cox P.A., Dalton V., Feinsinger P., Ingram M., Inouye D., Jones C.E., Kennedy K., Kevan P., Koopowitz H., Medellin R., Medellin-Morales S., Nabhan G.P., Pavlik B., Tepedino V., Torchio P. & Walker S. (1998) The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology*, 12, 8-17

Anagnostakis S.L. (1987) Chestnut Blight - the classical problem of an introduced pathogen. *Mycologia*, 79, 23-37

Andelman S.J. & Fagan W.F. (2000) Umbrellas and flagships: Efficient conservation surrogates or expensive mistakes? *Proceedings of the National Academy of Sciences of the United States of America*, 97, 5954-5959

Anderson P. (1995) Ecological restoration and creation: A review. *Biological Journal of the Linnean Society*, 56, 187-211

Anon (1995) Biodiversity: The UK Action Plan. In. HMSO, London

Arnold R.M. (1982) Pollination, predation and seed set in *Linaria Vulgaris* (Scrophulariaceae). *American Midland Naturalist*, 107, 360-369

Aronson J. & LeFloch E. (1996) Vital landscape attributes: Missing tools for restoration ecology. *Restoration Ecology*, 4, 377-387

Ashman T.L. & Stanton M. (1991) Seasonal variation in pollination dynamics of sexually dimorphic Sidalcea-Oregana ssp spicata (Malvaceae). *Ecology*, 72, 993-1003

Auld M., Davies S. & Pickess B. (1992a) Restoration of lowland heaths in dorset. *Conservation Review*, 6, 68-73

Baer B. & Schmid-Hempel P. (1999) Experimental variation in polyandry affects parasite loads and fitness in a bumblebee. *Nature*, 397, 151-154

Baer B. & Schmid-Hempel P. (2001) Unexpected consequences of polyandry for parasitism and fitness in the bumblebee, *Bombus terrestris*. *Evolution*, 55, 1639-1643

Balmford A., Bruner A., Cooper P., Costanza R., Farber S., Green R.E., Jenkins M., Jefferiss P., Jessamy V., Madden J., Munro K., Myers N., Naeem S., Paavola J., Rayment M., Rosendo S., Roughgarden J., Trumper K. & Turner R.K. (2002) Ecology - Economic reasons for conserving wild nature. *Science*, 297, 950-953

Barclay-Estrup P. & Gimingham C.H. (1969) Description and interpretation of cyclical processes in a heath community. 1. Vegetational change in relation to *Calluna* cycle. *Journal of Ecology*, 57, 737-&

Barclay-Estrup P. (1970) Description and interpretation of cyclical processes in a heath community. 2. Changes in biomass and shoot production during *Calluna* cycle. *Journal of Ecology*, 58, 243-&

Barclay-Estrup P. (1971) Description and interpretation of cyclical processes in a heath community. 3. Microclimate in relation to *Calluna* cycle. *Journal of Ecology*, 59, 143-&

Baron J.S., Poff N.L., Angermeier P.L., Dahm C.N., Gleick P.H., Hairston N.G., Jackson R.B., Johnston C.A., Richter B.D. & Steinman A.D. (2002) Meeting ecological and societal needs for freshwater. *Ecological Applications*, 12, 1247-1260

Barrett S.C.H. (2003) Mating strategies in flowering plants: the outcrossing-selfing paradigm and beyond. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, 358, 991-1004

Barthell J.F., Randall J.M., Thorp R.W. & Wenner A.M. (2001) Promotion of seed set in yellow star-thistle by honeybees: Evidence of an invasive mutualism. *Ecological Applications*, 11, 1870-1883

Bascompte J. & Jordano P. (2006) The Structure of plant-animal mutualistic etworks. In: *Ecological networks: linking structure to dynamics in food webs* (eds. Pascual M & Dunne JA), pp. 143-159. Oxford University Press

Bascompte J., Jordano P., Melian C.J. & Olesen J.M. (2003) The nested assembly of plant-animal mutualistic networks. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 9383-9387

Bennett A.F., Henein K. & Merriam G. (1994) Corridor use and the elements of corridor quality - Chipmunks and fencerows in a farmland mosaic. *Biological Conservation*, 68, 155-165

Benton T. (2000) *The Bumblebees of Essex*. Lopinga Books.

Bertness M.D. (1984) Habitat and community modification by an introduced herbivorous snail. *Ecology*, 65, 370-381

Bertness M.D. & Callaway R. (1994) Positive interactions in communities. *Trends in Ecology & Evolution*, 9, 191-193

Bertness M.D. & Leonard G.H. (1997) The role of positive interactions in communities: Lessons from intertidal habitats. *Ecology*, 78, 1976-1989

Bibby C.J. (1978) Conservation of Dartford Warbler on english lowland heaths - review. *Biological Conservation*, 13, 299-307

Bond W.J. (1994) Do Mutualisms Matter - Assessing the impact of pollinator and disperser disruption on plant extinction. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, 344, 83-90

Boucher D.H., James S. & Keeler K.H. (1982) The Ecology of mutualism. *Annual Review of Ecology and Systematics*, 13, 315-347

Bowers M.A. (1985) Bumblebee colonization, extinction, and reproduction in subalpine meadows in northeastern Utah. *Ecology*, 66, 914-927

Bradshaw A.D. (1987) Restoration: An acid test for ecology. In: *Restoration Ecology: A synthetic approach to ecological research* (eds. Jordan WR, Gilpin ME & Aber HJD), pp. 23-29. Cambridge University Press, U.K.

Brown J.H. (1995) Organisms and species as complex adaptive systems: linking the biology of populations with the physics of ecosystems. In: *Linking species and ecosystems* (eds. Jones CG & Lawton JH), pp. 16-24. Chapman and hall, New York

Brown M.J.F., Loosli R. & Schmid-Hempel P. (2000) Condition-dependent expression of virulence in a trypanosome infecting bumblebees. *Oikos*, 91, 421-427

Brualdi R.A. & Sanderson J.G. (1999) Nested species subsets, gaps, and discrepancy. *Oecologia*, 119, 256-264

Bruno J.F., Stachowicz J.J. & Bertness M.D. (2003) Inclusion of facilitation into ecological theory. *Trends in Ecology & Evolution*. 18. 119-125

Brussaard L. (1998) Soil fauna, guilds, functional groups and ecosystem processes. *Applied Soil Ecology*, 9, 123-135

Buchmann S.L. & Nabhan G.P. (1996) *The forgotten pollinators*. Island Press, Washington D.C., USA.

Byers J.E. & Noonburg E.G. (2003) Scale dependent effects of biotic resistance to biological invasion. *Ecology*, 84, 1428-1433

Cairns J. & Heckman J.R. (1996) Restoration ecology: The state of an emerging field. *Annual Review of Energy and the Environment*. 21, 167-189

Cane J.H. & Payne J.A. (1993) Regional, Annual, and Seasonal-Variation in Pollinator Guilds - Intrinsic traits of bees (Hymenoptera, Apoidea) underlie their patterns of abundance at vaccinium-Ashei (Ericaceae). *Annals of the Entomological Society of America*, 86, 577-588

Canning E.U. (1982) An evaluation of protozoal characteristics in relation to biological control of pests. In: *Parasites as Biological Control Agents* (eds. Anderson RM & Canning EU), pp. 119-149. Symposia of the British Society for Parasitology

Carder A.C. (1963) Control of yellow toadflax (*Linaria vulgaris*) in grass competition plus 2,4-D. *Weeds*, 11, 13-14

Cardinale B.J. & Palmer M.A. (2002) Disturbance moderates biodiversity-ecosystem function relationships: Experimental evidence from caddisflies in stream mesocosms. *Ecology*, 83, 1915-1927

Cardinale B.J., Palmer M.A. & Collins S.L. (2002) Species diversity enhances ecosystem functioning through interspecific facilitation. *Nature*, 415, 426-429

Carson R. (2002) *Silent Springs*. Houghton Mifflin Books.

Carvell C. (2002) Habitat use and conservation of bumblebees (*Bombus* spp.) under different grassland management regimes. *Biological Conservation*, 103, 33-49

Carvell C., Roy D.B., Smart S.M., Pywell R.F., Preston C.D. & Goulson D. (2006) Declines in forage availability for bumblebees at a national scale. *Biological Conservation*, 132, 481-489

Chapin F.S., Zavaleta E.S., Eviner V.T., Naylor R.L., Vitousek P.M., Reynolds H.L., Hooper D.U., Lavorel S., Sala O.E., Hobbie S.E., Mack M.C. & Diaz S. (2000) Consequences of changing biodiversity. *Nature*, 405, 234-242

Chapman M. (1931) *Animal Ecology*. McGraw-Hill, New York.

Chapman R.E., Wang J. & Bourke A.F.G. (2003) Genetic analysis of spatial foraging patterns and resource sharing in bumblebee pollinators. *Molecular Ecology*, 12, 2801-2808

Chapuis J.L., Bousses P. & Barnaud G. (1994) Alien mammals, impact and management in the french sub-antarctic islands. *Biological Conservation*, 67, 97-104

Chase J.M., Abrams P.A., Grover J.P., Diehl S., Chesson P., Holt R.D., Richards S.A., Nisbet R.M. & Case T.J. (2002) The interaction between predation and competition: a review and synthesis. *Ecology Letters*, 5, 302-315

Clements D.K. (1997) The enemy within: Conopid flies as parasitoids of bees and wasps in Britain. *British Wildlife*, 8, 310-315

Colla S.R., Otterstatter M.C., Gegear R.J. & Thomson J.D. (2006) Plight of the bumble bee: Pathogen spillover from commercial to wild populations. *Biological Conservation*, 129, 461-467

Cone D.K., Marcogliese D.J. & Watt W.D. (1993) Metazoan Parasite Communities of Yellow Eels (*Anguilla rostrata*) in Acidic and Limed Rivers of Nova-Scotia. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, 71, 177-184

Constible J.M., Sweitzer R.A., Van Vuren D.H., Schuyler P.T. & Knapp D.A. (2005) Dispersal of non-native plants by introduced bison in an island ecosystem. *Biological Invasions*, 7, 699-709

Corbet S.A. (1987) More bees make better crops. *New Scientist*, 115, 40-43

Corbet S.A. (2000) Conserving compartments in pollination webs. *Conservation Biology*, 14, 1229-1231

Corbet S.A., Fussell M., Ake R., Fraser A., Gunson C., Savage A. & Smith K. (1993) Temperature and the Pollinating Activity of Social Bees. *Ecological Entomology*, 18, 17-30

Corbet S.A., Williams I.H. & Osborne J.L. (1991) Bees and the pollination of crops and wild flowers in the European Community. *Bee World*, 72, 47-59

Costanza R., d'Arge R., de Groot R., Farber S., Grasso M., Hannon B., Limburg K., Naeem S., O'Neill R.V., Paruelo J., Raskin R.G., Sutton P. & van den Belt M. (1997) The value of the world's ecosystem services and natural capital. 387, 253-260

Crawley M.L. (1987) What makes a community invasible? *In: Colonisation, succession and stability. Pp 429-453*. Blackwell, Oxford.

Daily G.C. (1997) Introduction: what are ecosystem services. In: *Nature's Services: Societal dependence on natural ecosystems.* (ed. Daily G.C.), pp. 1-10. Island Press, Washington D.C.

D'Antonio C.M. & J.C. Chambers (2006) Using ecological theory to manage or restore ecosystems affected by invasive plant species. In: *Foundations of restoration ecology* (eds. Falk D.A., Palmer M.A. & Zedler, L.), pp. 260-279. Island Press

D'Antonio C.M. & Vitousek P.M. (1992) Biological invasions by exotic grasses, the grass fire cycle, and global change. *Annual Review of Ecology and Systematics*, 23, 63-87

Darvill B., Knight M.E. & Goulson D. (2004) Use of genetic markers to quantify bumblebee foraging range and nest density. *Oikos*, 107, 471-478

Darwin C. (1859) *The Origin of Species*. John Murrey, London.

Davis M.A., Thompson K. & Grime J.P. (2005) Invasibility: the local mechanism driving community assembly and species diversity. *Ecography*, 28, 696-704

Dejong T.J., Waser N.M. & Klinkhamer P.G.L. (1993) Geitonogamy - the neglected side of selfing. *Trends in Ecology & Evolution*, 8, 321-325

Dias B.S.F., Raw A. & Imperatri-Fonseca V.L. (1999) Report on the recommendations of the workshop on the conservation and sustainable use of pollinators in agriculture, with emphasis on bees. In. International Pollinators Initiative: Sao Paulo Declaration on Pollinators., Brazilian Ministry of the Environment (MMA)

Dicks L.V., Corbet S.A. & Pywell R.F. (2002) Compartmentalization in plant-insect flower visitor webs. *Journal of Animal Ecology*, 71, 32-43

Didham R.K., Lawton J.H., Hammond P.M. & Eggleton P. (1998) Trophic structure stability and extinction dynamics of beetles (Coleoptera) in tropical forest fragments. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, 353, 437-451

Diekotter T., Walther-Hellwig K., Conradi M., Suter M. & Frankl R. (2006) Effects of landscape elements on the distribution of the rare bumblebee species *Bombus muscorum* in an agricultural landscape. *Biodiversity and Conservation*, 15, 57-68

Diez J. (2005) Invasion biology of Australian ectomycorrhizal fungi introduced with eucalypt plantations into the Iberian Peninsula. *Biological Invasions*, 7, 3-15

Dobson A.P., Bradshaw A.D. & Baker A.J.M. (1997) Hopes for the future: Restoration ecology and conservation biology. *Science*, 277, 515-522

Dobson A.P. & Hudson P.J. (1986) Parasites, disease and the structure of ecological communities. *Trends in Ecology & Evolution*, 1, 11-15

Dobson A.P., Lafferty K.D. & Kuris A.M. (2006) Parasites and food webs. In: *Ecological networks: linking structure to dynamics in food webs.* (eds. Pascual, M. & Dunne, J.A.), pp. 119-135. Oxford University Press, Oxford

Donaldson J., Nanni I., Zachariades C., Kemper J. & Thompson J.D. (2002) Effects of habitat fragmentation on pollinator diversity and plant reproductive success in renosterveld shrublands of South Africa. *Conservation Biology*, 16, 1267-1276

Drake J.A., Mooney, H.A., Di Castri, F., Groves, R.H., Kruger, F.J., Rejmanek, M. & Williamson, M (1989) *Biological Invasions: A Global Perspective*. Wiley, Chichester, USA.

Dramstad W.E. (1996) Do bumblebees (Hymenoptera: Apidae) really forage close to their nests? *Journal of Insect Behavior*, 9, 163-182

Dunne J.A., Williams R.J. & Martinez N.D. (2002) Network structure and biodiversity loss in food webs: robustness increases with connectance. *Ecology Letters*, 5, 558-567

Durrer S. & Schmid-Hempel P. (1994) Shared Use of Flowers Leads to Horizontal Pathogen Transmission. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 258, 299-302

Durrer S. & Schmid-Hempel P. (1995) Parasites and the regional distribution of bumblebee species. *Ecography*, 18, 114-122

Dytham C. (2003) *Choosing and Using Statistics, a Biologist's Guide*. Blackwell Science.

Ebert D. & Hamilton W.D. (1996) Sex against virulence: The coevolution of parasitic diseases. *Trends in Ecology & Evolution*, 11, A79-A82

Edwards M. & Williams P.H. (2004) Where have all the bumblebees gone, and could they ever return? *British Wildlife*, 15, 305-312

Ehrenfeld J.G. (2000) Defining the limits of restoration: The need for realistic goals. *Restoration Ecology*, 8, 2-9

Ellis J.S., Knight M.E., Darvill B. & Goulson D. (2006) Extremely low effective population sizes, genetic structuring and reduced genetic diversity in a threatened bumblebee species, *Bombus sylvarum* (Hymenoptera : Apidae). *Molecular Ecology*, 15, 4375-4386

Elton C.S. (1958) *The Ecology of Invasions by Animals and Plants*. University of Chicago Press, Chicago, USA.

Esch G.W. & Fernandez J.C. (1993) *A Functional Biology of Parasitism. Ecological and Evolutionary Implications*. Chapman and Hall, London.

Falk D.A., Palmer M.A. & Zedler L. (2006) *Foundations of restoration ecology*. Island Press.

Fishbein M. & Venable D.L. (1996) Diversity and temporal change in the effective pollinators of *Asclepias tuberosa*. *Ecology*, 77, 1061-1073

Flatt T. & Scheuring I. (2004) Stabilizing factors interact in promoting host-parasite coexistence. *Journal of Theoretical Biology*, 228, 241-249

Floerl O., Pool T.K. & Inglis G.J. (2004) Positive interactions between nonindigenous species facilitate transport by human vectors. *Ecological Applications*, 14, 1724-1736

Fonseca C.R. & Ganade G. (1996) Asymmetries, compartments and null interactions in an Amazonian ant-plant community. *Journal of Animal Ecology*, 65, 339-347

Fonseca C.R. & Ganade G. (2001) Species functional redundancy, random extinctions and the stability of ecosystems. *Journal of Ecology*, 89, 118-125

Forup M.L. (2003) *The Restoration of Plant-Pollinator Interactions*. Ph.D. Thesis, University of Bristol, UK.

Forup M.L., Henson K.S.E. & Memmott J. (*in press*) The restoration of ecological interactions: plant-pollinator networks on ancient and restored heathlands. *Journal of Applied Ecology*

Forup M.L. & Memmott J. (2005a) The restoration of plant-pollinator interactions in hay meadows. *Restoration Ecology*, 13, 265-274

Forup M.L. & Memmott J. (2005b) The relationship between the abundances of bumblebees and honeybees in a native habitat. *Ecological Entomology*, 30, 47-57

Freckman D.W., Blackburn T.H., Brussaard L., Hutchings P., Palmer M.A. & Snelgrove P.V.R. (1997) Linking biodiversity and ecosystem functioning of soils and sediments. *Ambio*, 26, 556-562

Freeland W.J. & Boulton W.J. (1992) Coevolution of food webs - parasites, predators and plant secondary compounds. *Biotropica*, 24, 309-327

Gathmann A. & Tscharntke T. (2002) Foraging ranges of solitary bees. *Journal of Animal Ecology*, 71, 757-764

Gegear R.J., Otterstatter M.C. & Thomson J.D. (2005) Does parasitic infection impair the ability of bumblebees to learn flower-handling techniques? *Animal Behaviour*, 70, 209-215

Gegear R.J., Otterstatter M.C. & Thomson J.D. (2006) Bumble-bee foragers infected by a gut parasite have an impaired ability to utilize floral information. *Proceedings of the Royal Society B-Biological Sciences*, 273, 1073-1078

Glasser J.W. (1982) On the causes of temporal change in communities - modification of the biotic environment. *American Naturalist*, 119, 375-390

Godfray H.C.J. (1994) *Parasitoids*. Princeton University Press, USA.

Goka K., Okabe K. & Yoneda M. (2006) Worldwide migration of parasitic mites as a result of bumblebee commercialization. *Population Ecology*, 48, 285-291

Gotelli N.J. & Simberloff D. (1987) The distribution and abundance of tallgrass prairie plants - a test of the core-satellite hypothesis. *American Naturalist*, 130, 18-35

Goulson D. (2003) *Bumblebees: Behaviour and Ecolgoy*. Oxford University Press, Oxford, UK.

Goulson D., Hanley M.E., Darvill B. & Ellis J.S. (2006) Biotope associations and the decline of bumblebees (*Bombus* spp.). *Journal of Insect Conservation*, 10, 95-103

Goulson D., Hanley M.E., Darvill B., Ellis J.S. & Knight M.E. (2005) Causes of rarity in bumblebees. *Biological Conservation*, 122, 1-8

Grosholz E.D. (2005) Recent biological invasion may hasten invasional meltdown by accelerating historical introductions. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 1088-1091

Gross R.S. & Werner P.A. (1983) Relationships among flowering phenology, insect visitors, and seed-set of individuals - experimental studies on 4 co- Occurring species of goldenrod (Solidago, Compositae). *Ecological Monographs*, 53, 95-117

Hairston N.G., Smith F.E. & Slobodkin L.B. (1960) Community structure, population control, and competition. *American Naturalist*, 94, 421-425

Hallett H.M. (1928) The Hymneoptera Aculeata of Glamorgan. *Transactions of the Cardiff Naturalists' Society*, 60, 33-67

Handel S.N. (1997) *The role of plant-animal mutualisms in the design and restoration of natural communities*. Cambridge University Press, Cambridge.

Hanski I. (1982) Dynamics of regional distribution - the core and satellite species hypothesis. *Oikos*, 38, 210-221

Hawken P. & Lovins A.B. (1999) *Natural capitalism: the next industrial revolution*. Earthscan Publications Ltd., London.

Heal G. (2000) *Nature and the Marketplace: Capturing the Value of Ecosystem Services*. Island Press, Covelo, CA.

Heinrich B. (1979) *Bumblebee Economics*. Harvard University Press, Cambridge, MA.

Helms K.R. & Vinson S.B. (2003) Apparent facilitation of an invasive mealybug by an invasive ant. *Insectes Sociaux*, 50, 403-404

Herrera C.M. (1987) Components of pollinator quality – comparative analysis of a diverse insect assemblage. *Oikos*, 50, 79-90

Herrera C.M. (1988) Variation in mutualisms - the spatio-temporal mosaic of a pollinator assemblage. *Biological Journal of the Linnean Society*, 35, 95-125

Heywood V.H. & Iriondo J.M. (2003) Plant conservation: old problems, new perspectives. *Biological Conservation*, 113, 321-335

Higgins S.I., Turpie J.K., Costanza R., Cowling R.M., LeMaitre D.C., Marais C. & Midgley G.F. (1997) An ecological economic simulation model of mountain fynbos ecosystems - Dynamics, valuation and management. *Ecological Economics*, 22, 155-169

Higgs E.S. (1997) What is good ecological restoration? *Conservation Biology*, 11, 338-348

Hobbs R.J. (1992) The role of corridors in conservation - solution or bandwagon. *Trends in Ecology & Evolution*, 7, 389-392

Hobbs R.J. & Huenneke L.F. (1992) Disturbance, diversity, and invasion - implications for conservation. *Conservation Biology*, 6, 324-337

Hobbs R.J. & Norton D.A. (1996) Towards a conceptual framework for restoration ecology. *Restoration Ecology*, 4, 93-110

Hodkinson D.J. & Thompson K. (1997) Plant dispersal: the role of man. *Journal of Applied Ecology*, 34, 1484-1496

Hoffmann J.H. (1990) Interactions between 3 weevil species in the biocontrol of *Sesbania punicea* (Fabaceae) - the role of simulation models in evaluation. *Agriculture Ecosystems & Environment*, 32, 77-87

Holt R.D. (1996) Food webs in space: an island biogeographic perspective. In: *Food webs*. (eds. Polis GA & Winemiller KO), pp. 313-323. Chapman and Hall, New York

Holt R.D., Lawton J.H., Polis G.A. & Martinez N.D. (1999) Trophic rank and the species-area relationship. *Ecology*, 80, 1495-1504

Horwitz P. & Wilcox B.A. (2005) Parasites, ecosystems and sustainability: an ecological and complex systems perspective. *International Journal for Parasitology*, 35, 725-732

Huspeni T.C. & Lafferty K.D. (2004) Using larval trematodes that parasitize snails to evaluate a saltmarsh restoration project. *Ecological Applications*, 14, 795-804

Huxham M., Beaney S. & Raffaelli D. (1996) Do parasites reduce the chances of triangulation in a real food web? *Oikos*, 76, 284-300

Huxham, M., Raffaelli, D. & Pike, A. (1995) Parasites and food web patterns. The Journal of Animal Ecology 64 (2), 168-176

Imhoof B. & Schmid-Hempel P. (1998) Single-clone and mixed-clone infections versus host environment in *Crithidia bombi* infecting bumblebees. *Parasitology*, 117, 331-336

Imhoof B. & Schmid-Hempel P. (1999) Colony success of the bumble bee, *Bombus terrestris*, in relation to infections by two protozoan parasites, *Crithidia bombi* and *Nosema bombi*. *Insectes Sociaux*, 46, 233-238

Ings T.C., Schikora J. & Chittka L. (2005) Bumblebees, humble pollinators or assiduous invaders? A population comparison of foraging performance in *Bombus terrestris*. *Oecologia*, 144, 508-516

Ings T.C., Ward N.L. & Chittka L. (2006) Can commercially imported bumble bees out-compete their native conspecifics? *Journal of Applied Ecology*, 43, 940-948

Irwin R.E. & Maloof J.E. (2002) Variation in nectar robbing over time, space, and species. *Oecologia*, 133, 525-533

Ives A.R. & Cardinale B.J. (2004) Food-web interactions govern the resistance of communities after non-random extinctions. *Nature*, 429, 174-177

Ives A.R., Klug J.L. & Gross K. (2000) Stability and species richness in complex communities. *Ecology Letters*, 3, 399-411

Janzen D.H. (1974) The deflowering of Central America. *Natural History*, 83, 48-53

Jennersten O. (1988) Pollination in *Dianthus deltoides* (Caryophyllaceae) - Effects of habitat fragmentation on visitation and seed set. *Conservation Biology*, 2, 359-366

Jiang L. & Morin P.J. (2004) Productivity gradients cause positive diversity-invasibility relationships in microbial communities. *Ecology Letters*, 7, 1047-1057

Johansen C.A. (1977) Pesticides and pollinators. *Annual Review of Entomology*, 22, 177-192

Jordano P. (1987) Patterns of mutualistic interactions in pollination and seed dispersal - connectance, dependence asymmetries, and coevolution. *American Naturalist*, 129, 657-677

Jules E.S. & Shahani P. (2003) A broader ecological context to habitat fragmentation: Why matrix habitat is more important than we thought. *Journal of Vegetation Science*, 14, 459-464

Kareiva P. (1996) Developing a predictive ecology for non-indigenous species and ecological invasions. *Ecology*, 77, 1651-1652

Kaufmann S., McKey D.B., Hossaert-McKey M. & Horvitz C.C. (1991) Adaptations for a two-phase seed dispersal system involving vertebrates and ants in a hemiepiphytic fig (*Ficus microcarpa*: Moraceae). *American Journal of Botany*, 78, 971-977

Kay Q.O.N. (1985) Hermaphrodites and subhermaphrodites in a reputedly dioecious plant, *Cirsium arvense*(L) Scop. *New Phytologist*, 100, 457-472

Kearns C.A. (2001) North American, dipteran pollinators: Assessing their value and conservation status. *Conservation Ecology*, 5

Kearns C.A. & Inouye D.S. (1997) Pollinators, flowering plants, and conservation biology - Much remains to be learned about pollinators and plants. *Bioscience*, 47, 297-307

Kearns C.A., Inouye D.W. & Waser N.M. (1998) Endangered mutualisms: The conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics*, 29, 83-112

Kells A.R. & Goulson D. (2003) Preferred nesting sites of bumblebee queens (Hymenoptera : Apidae) in agroecosystems in the UK. *Biological Conservation*, 109, 165-174

Kirby P. (1992) *Habitat management for invertebrates.* Royal Society for the Protection of Birds, Sandy, U.K.

Klironomos J.N. (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, 417, 67-70

Knight M.E., Martin A.P., Bishop S., Osborne J.L., Hale R.J., Sanderson A. & Goulson D. (2005) An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species. *Molecular Ecology*, 14, 1811-1820

Knops J.M.H., Tilman D., Haddad N.M., Naeem S., Mitchell C.E., Haarstad J., Ritchie M.E., Howe K.M., Reich P.B., Siemann E. & Groth J. (1999) Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. *Ecology Letters*, 2, 286-293

Kolar C.S. & Lodge D.M. (2001) Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution*, 16, 199-204

Komonen A., Penttila R., Lindgren M. & Hanski I. (2000) Forest fragmentation truncates a food chain based on an old-growth forest bracket fungus. *Oikos*, 90, 119-126

Kondoh M. (2003) Foraging adaptation and the relationship between food-web complexity and stability. *Science*, 299, 1388-1391

Kraus B. & Page R.E. (1998) Parasites, pathogens, and polyandry in social insects. *American Naturalist*, 151, 383-391

Krause A.E., Frank K.A., Mason D.M., Ulanowicz R.E. & Taylor W.W. (2003) Compartments revealed in food-web structure. *Nature*, 426, 282-285

Kreyer D., Oed A., Walther-Hellwig K. & Frankl R. (2004) Are forests potential landscape barriers for foraging bumblebees? Landscape scale experiments

with *Bombus terrestris* agg. and *Bombus pascuorum* (Hymenoptera, Apidae). *Biological Conservation*, 116, 111-118

Kruess A. & Tscharntke T. (1994) Habitat fragmentation, species loss, and biological control. *Science*, 264, 1581-1584

Kruess A. & Tscharntke T. (2000) Species richness and parasitism in a fragmented landscape: experiments and field studies with insects on *Vicia sepium*. *Oecologia*, 122, 129-137

Kwak M., Brand C., Kremer P. & Boerrigter E. (1991) Visitation, flight distances and seed set in populations of the rare species *Phyteuma nigrum* (Campanulaceae). *Acta Horticulturae 288*, 288, 303-307

Lafferty K.D. (1997) Environmental parasitology: What can parasites tell us about human impacts on the environment? *Parasitology Today*, 13, 251-255

Lafferty K.D. (2003) Is disease increasing or decreasing, and does it impact or maintain biodiversity? *Journal of Parasitology*, 89, S101-S105

Lafferty K.D., Dobson A.P. & Kuris A.M. (2006) Parasites dominate food web links. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 11211-11216

Lalonde R.G. & Roitberg B.D. (1994) Mating System, Life-History, and Reproduction in Canada Thistle (*Cirsium arvense* Asteraceae). *American Journal of Botany*, 81, 21-28

Lambeck R.J. (1997) Focal species: A multi-species umbrella for nature conservation. *Conservation Biology*, 11, 849-856

Lawton J.H. & Brown V.K. (1993) Redundancy in ecosystems. In: *Biodiversity and Ecosystem Function* (eds. Schulze ED & Mooney HA), pp. 255-268. Springer, New York

Lennartsson T. (2002) Extinction thresholds and disrupted plant-pollinator interactions in fragmented plant populations. *Ecology*, 83, 3060-3072

Lenski R.E. & May R.M. (1994) The evolution of virulence in parasites and pathogens - Reconciliation between 2 competing hypotheses. *Journal of Theoretical Biology*, 169, 253-265

Levin S.A. (1998) Ecosystems and the biosphere as complex adaptive systems. *Ecosystems*, 1, 431-436

Levine J.M. (2000) Species diversity and biological invasions: Relating local process to community pattern. *Science*, 288, 852-854

Levine J.M. & D'Antonio C.M. (1999) Elton revisited: a review of evidence linking diversity and invasibility. *Oikos*, 87, 15-26

Levine J.M. & D'Antonio C.M. (2003) Forecasting biological invasions with increasing international trade. *Conservation Biology*, 17, 322-326

Levine J.M., Vila M., D'Antonio C.M., Dukes J.S., Grigulis K. & Lavorel S. (2003) Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 270, 775-781

Lewis O.T., Memmott J., Lasalle J., Lyal C.H.C., Whitefoord C. & Godfray H.C.J. (2002) Structure of a diverse tropical forest insect-parasitoid community. *Journal of Animal Ecology*, 71, 855-873

Liley D. & Clarke R.T. (2003) The impact of urban development and human disturbance on the numbers of nightjar *Caprimulgus europaeus* on heathlands in Dorset, England. *Biological Conservation*, 114, 219-230

Lindenmayer D.B., Manning A.D., Smith P.L., Possingham H.P., Fischer J., Oliver I. & McCarthy M.A. (2002) The focal-species approach and landscape restoration: a critique. *Conservation Biology*, 16, 338-345

Lipa J.J. & Triggiani O. (1988) Crithidia-Bombi Sp N. A Flagellated Parasite of a Bumblebee *Bombus terrestris* L (Hymenoptera, Apidae). *Acta Protozoologica*, 27, 287-&

Liu H.J., Macfarla.Rp & Pengelly D.H. (1974) *Mattesia bombi* N-Sp (Neogregarinida - Ophrocystidae), a Parasite of *Bombus* (Hymenoptera - Apidae). *Journal of Invertebrate Pathology*, 23, 225-231

Lloyd D.G. & Myall A.J. (1976) Sexual Dimorphism in *Cirsium arvense* L Scop. *Annals of Botany*, 40, 115-&

Lonsdale W.M. (1999) Global patterns of plant invasions and the concept of invasibility. *Ecology*, 80, 1522-1536

Loreau M., Naeem S., Inchausti P., Bengtsson J., Grime J.P., Hector A., Hooper D.U., Huston M.A., Raffaelli D., Schmid B., Tilman D. & Wardle D.A. (2001) Ecology - Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science*, 294, 804-808

Losey J.E. & Vaughan M. (2006) The economic value of ecological services provided by insects. *Bioscience*, 56, 311-323

Lovei G.L. (1997) Biodiversity - Global change through invasion. *Nature*, 388, 627-628

Lundberg S. & Ingvarsson P.K. (1998) Population dynamics of resource limited plants and their pollinators. *Theoretical Population Biology*, 54, 44-49

Macfarlane R.P. & Griffin R.P. (1990) New Zealand distribution and seasonal incidence of the nematode, *Sphaerularia bombi* Dufour, a parasite of bumble bees. *New Zealand Journal of Zoology*, 17, 191-199

Macfarlane R.P., Lipa J.J. & Liu H.J. (1995) Bumble bee pathogens and internal enemies. *Bee World*, 76, 130-148

Mack R.N. (2003) Plant naturalizations and invasions in the eastern United States: 1634-1860. *Annals of the Missouri Botanical Garden*, 90, 77-90

Mack R.N., Simberloff D., Lonsdale W.M., Evans H., Clout M. & Bazzaz F.A. (2000) Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications*, 10, 689-710

Magurran A.L. (2004) *Measuring Biological Diversity*. Blackwell Oxford, UK.

Marcogliese D.J. (2003) Food webs and biodiversity: are parasites the missing link? *Journal of Parasitology*, 89, S106-S113

Marcogliese D.J. (2005) Parasites of the superorganism: Are they indicators of ecosystem health? *International Journal for Parasitology*, 35, 705-716

Marcogliese D.J. & Cone D.K. (1997) Food webs: A plea for parasites. *Trends in Ecology & Evolution*, 12, 320-325

Martinez N.D. (1991) Artifacts or Attributes - Effects of Resolution on the Little-Rock Lake Food Web. *Ecological Monographs*, 61, 367-392

Martinez N.D. (1992) Constant Connectance in Community Food Webs. *American Naturalist*, 139, 1208-1218

Maschinski J. (2006) Implications of population dynamics and metapopulation theory for restoration. In: *Foundations of restoration ecology* (eds. Falk DA, Palmer MA & Zedler L), pp. 59-87. Island Press

Mawdsley N.A. & Stork N.E. (1995) Species Extinctions in insects: ecological and biogeographical considerations. In: *Insects in a Changing Environment* (eds. Harrington R & Stork NE), pp. 321-369. Academic Press, London UK

McCann K., Hastings A. & Huxel G.R. (1998) Weak trophic interactions and the balance of nature. *Nature*, 395, 794-798

McCoy E.D. & Mushinsky H.R. (2002) Measuring the success of wildlife community restoration. *Ecological Applications*, 12, 1861-1871

McEvoy P.B. & Coombs E.M. (1999) Biological control of plant invaders: Regional patterns, field experiments, and structured population models. *Ecological Applications*, 9, 387-401

McIvor C.A. & Malone L.A. (1995) *Nosema bombi*, a Microsporidian Pathogen of the Bumble Bee *Bombus terrestris* (L). *New Zealand Journal of Zoology*, 22, 25-31

MEA (2005) Ecosystems and human well-being: A synthesis. In: *Millennium Ecosystem Assessment*, Island Press, Washington D.C.

Melian C.J. & Bascompte J. (2004) Food web cohesion. *Ecology*, 85, 352-358

Memmott J. (1999) The structure of a plant-pollinator food web. *Ecology Letters*, 2, 276-280

Memmott J., Alonso D., Berlow E.L., Dobson A., Dunne J.A., Sole R. & Weitz J. (2006) Biodiversity loss and ecological network structure. In: *Ecological networks: linking structure to dynamics in food webs* (eds. Pascual M & Dunne JA), pp. 325-347. Oxford University Press

Memmott J., R.Gibson, Caravalheiro L.G., Henson K., Heleno R.H., Mikel M.L. & Pearce S. (*In press*) The conservation of ecological interactions. In: *Insect Conservation Biology* (eds. Stewart AJA, New TR & Lewis OT), pp. 226 - 244. The Royal Entomological Society, London, U.K.

Memmott J. & Waser N.M. (2002) Integration of alien plants into a native flower-pollinator visitation web. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 269, 2395-2399

Memmott J., Waser N.M. & Price M.V. (2004) Tolerance of pollination networks to species extinctions. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 271, 2605-2611

Menge B.A. & Sutherland J.P. (1976) Species diversity gradients - synthesis of roles of predation, competition, and temporal heterogeneity. *American Naturalist*, 110, 351-369

Menninger H.L. & Palmer M.A. (2006) Restoring ecological communities: From theory to practice. In: *Foundations of restoration ecology* (eds. Falk DA, Palmer MA & Zedler L), pp. 88-112. Island Press

Michener W.K. (1997) Quantitatively evaluating restoration experiments: Research design, statistical analysis, and data management considerations. *Restoration Ecology*, 5, 324-337

Miller J.C. (1993) Insect natural history, multispecies interactions and biodiversity in ecosystems. *Biodiversity and Conservation*, 2, 233-241

Minchella D.J. & Scott M.E. (1991) Parasitism - a cryptic determinant of animal community structure. *Trends in Ecology & Evolution*, 6, 250-254

Minckley R.L., Cane J.H., Kervin L. & Roulston T.H. (1999) Spatial predictability and resource specialization of bees (Hymenoptera : Apoidea) at a superabundant, widespread resource. *Biological Journal of the Linnean Society*, 67, 119-147

Mitchell C.E., Agrawal A.A., Bever J.D., Gilbert G.S., Hufbauer R.A., Klironomos J.N., Maron J.L., Morris W.F., Parker I.M., Power A.G., Seabloom E.W., Torchin M.E. & Vazquez D.P. (2006) Biotic interactions and plant invasions. *Ecology Letters*, 9, 726-740

Mitchell R.J., Marrs R.H., Le Duc M.G. & Auld M.H.D. (1999) A study of the restoration of heathland on successional sites: changes in vegetation and soil chemical properties. *Journal of Applied Ecology*, 36, 770-783

Mitsuhata M. & Ono M. (1996) Hybridization between Japanese and european bumblebees (*Bombus* spp.). In: *Summaries of the 7th international pollination symposium, Canada.*

Montalvo A.M., Williams S.L., Rice K.J., Buchmann S.L., Cory C., Handel S.N., Nabhan G.P., Primack R. & Robichaux R.H. (1997) Restoration biology: A population biology perspective. *Restoration Ecology*, 5, 277-290

Montgomery B.R., Kelly D., Robertson A.W. & Ladley J.J. (2003) Pollinator behaviour, not increased resources, boosts seed set on forest edges in a New Zealand Loranthaceous mistletoe. *New Zealand Journal of Botany*, 41, 277-286

Montoya J.M., Rodriguez M.A. & Hawkins B.A. (2003) Food web complexity and higher-level ecosystem services. *Ecology Letters*, 6, 587-593

Moore N.W. (1962) The heaths of Dorset and their conservation. *Journal of Ecology*. 50, 369-391

Moore R.J. (1975) Biology of Canadian Weeds .13. *Cirsium arvense*(L) Scop. *Canadian Journal of Plant Science*, 55, 1033-1048

Morales C.L. & Aizen M.A. (2002) Does invasion of exotic plants promote invasion of exotic flower visitors? A Case Study from the Temperate Forests of the Southern Andes. *Biological Invasions*, Volume 4, 87-100

Morales C.L. & Aizen M.A. (2006) Invasive mutualisms and the structure of plant-pollinator interactions in the temperate forests of north-west Patagonia, Argentina. *Journal of Ecology*, 94, 171-180

Morand S., Manning S.D. & Woolhouse M.E.J. (1996) Parasite-host coevolution and geographic patterns of parasite infectivity and host susceptibility. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 263, 119-128

Morin P.J. (1999) *Community Ecology*. Blackwell Science. Massachusetts, USA.

Morris R.J., Lewis O.T. & Godfray H.C.J. (2004) Experimental evidence for apparent competition in a tropical forest food web. *Nature*, 428, 310-313

Morris W.F. (2003) Which mutualists are most essential? Buffering of plant reproduction against the extinction of pollinators. In: *The Importance of Species: Perspectives on Expendability and Triage* (eds. Kareiva P & Levin SA), pp. 260-280. Princeton University Press, Princeton, New York

Mouillot, D., George-Nascimento, M. & Poulin, R. (2005) Richness, structure and functioning in metazoan parasite communities. Oikos 109, 447-460

Mouritsen K.N. & Poulin R. (2002) Parasitism, community structure and biodiversity in intertidal ecosystems. *Parasitology*, 124, S101-S117

Müller C.B., Blackburn T.M. & Schmid-Hempel P. (1996) Field evidence that host selection by conopid parasitoids is related to host body size. *Insectes Sociaux*, 43, 227-233

Murcia C. (1995) Edge effects in fragmented forests - Implications for conservation. *Trends in Ecology & Evolution*, 10, 58-62

Nadeau L.B., Dale M.R.T. & King J.R. (1991) The development of spatial pattern in shoots of *Linaria vulgaris* (Scrophulariaceae) growing on fallow land or in a barley crop. *Canadian Journal of Botany-Revue Canadienne De Botanique*, 69, 2539-2544

Naeem S. (1998) Species redundancy and ecosystem reliability. *Conservation Biology*, 12, 39-45

Naeem S. (2002) Ecosystem consequences of biodiversity loss: The evolution of a paradigm. *Ecology*, 83, 1537-1552

Naeem S., Knops J.M.H., Tilman D., Howe K.M., Kennedy T. & Gale S. (2000) Plant diversity increases resistance to invasion in the absence of covarying extrinsic factors. *Oikos*, 91, 97-108

Neal P.R. (1998) Pollinator restoration. *Trends in Ecology & Evolution*, 13, 132-133

Niwa S., Iwano H., Asada S., Matsuura M. & Goka K. (2004) A microsporidian pathogen isolated from a colony of the European bumblebee, *Bombus terrestris*, and infectivity on Japanese bumblebee. *Japanese Journal of Applied Entomology and Zoology*, 48, 60-64

Norberg J. (1999) Linking Nature's services to ecosystems: some general ecological concepts. *Ecological Economics*, 29, 183-202

Nowak M.A. & May R.M. (1994) Superinfection and the evolution of parasite virulence. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 255, 81-89

NRC (1992) Committee on restoration of aquatic ecosystems - science, technology, and public policy. In. National Research Council USA, National Academy Press, Washington DC.

Nuzzo V. (1997) Element Stewardship Abstract for *Circium arvense*. In: *The Nature Conservancy*

O'Dowd D.J., Green P.T. & Lake P.S. (2003) Invasional 'meltdown' on an oceanic island. *Ecology Letters*, 6, 812-817

Olesen J.M., Eskildsen L.I. & Venkatasamy S. (2002) Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. *Diversity and Distributions*, 8, 181-192

Ormerod S.J. (2003) Restoration in applied ecology: editor's introduction. *Journal of Applied Ecology*, 40, 44-50

Osborne J.L., Williams I.H. & Corbet S.A. (1991) Bees, pollination and habitat change in the european community. *Bee World*, 72, 99-116

Osenburg C.W., Bolker B.M., White J.S., St. Mary C.M. & Shima J.S. (2006) Stastistical issues and study design in ecological restorations: Lessons Learned from Marine Reserves. In: *Foundations of restoration ecology* (eds. Falk DA, Palmer MA & Zedler L), pp. 280-302. Island Press

Otterstatter M.C. (2004) Patterns of parasitism among conopid flies parasitizing bumblebees. *Entomologia Experimentalis Et Applicata*, 111, 133-139

Otterstatter M.C. & Whidden T.L. (2004) Patterns of parasitism by tracheal mites (*Locustacarus buchneri*) in natural bumble bee populations. *Apidologie*, 35, 351-357

Otterstatter M.C., Gegear R.J., Colla S.R. & Thomson J.D. (2005) Effects of parasitic mites and protozoa on the flower constancy and foraging rate of bumble bees. *Behavioral Ecology and Sociobiology*, 58, 383-389

Paine R.T. (1966) Food web complexity and species diversity. *American Naturalist*, 100, 65-&

Paine R.T. (1969) A note on trophic complexity and community stability. *American Naturalist*, 103, 91-&

Paine R.T. (1980) Food Webs - linkage, interaction strength and community infrastructure - the 3rd Tansley Lecture. *Journal of Animal Ecology*, 49, 667-685

Paine R.T. (1988) Road maps of interactions or grist for theoretical development? *Ecology*, 69, 1648-1654

Paine R.T. (1992) Food-Web analysis through field measurement of per-capita interaction strength. *Nature*, 355, 73-75

Palmer M., Bernhardt E., Chornesky E., Collins S., Dobson A., Duke C., Gold B., Jacobson R., Kingsland S., Kranz R., Mappin M., Martinez M.L., Micheli F., Morse J., Pace M., Pascual M., Palumbi S., Reichman O.J., Simons A., Townsend A. & Turner M. (2004) Ecology for a crowded planet. *Science*, 304, 1251-1252

Palmer M.A., Ambrose R.F. & Poff N.L. (1997) Ecological theory and community restoration ecology. *Restoration Ecology*, 5, 291-300

Palmer M.A., Falk D.A. & Zedler J.B. (2006) Ecological theory and restoration ecology. In: *Foundations of restoration ecology* (eds. Falk DA, Palmer MA & Zedler L), pp. 1-10. Island Press

Parker I.M., Simberloff D., Lonsdale W.M., Goodell K., Wonham M., Kareiva P.M., Williamson M.H., Holle B.V., Moyle P.B., Byers J.E. & Goldwasser L. (1999) Impact: Toward a framework for understanding the ecological effects of invaders. *Biological Invasions*, 1, 3-19

Parker J.D., Burkepile D.E. & Hay M.E. (2006) Opposing effects of native and exotic herbivores on plant invasions. *Science*, 311, 1459-1461

Parker V.T. (1997) The scale of successional models and restoration objectives. *Restoration Ecology*, 5, 301-306

Passell H.D. (2000) Recovery of bird species in minimally restored Indonesian tin strip mines. *Restoration Ecology*, 8, 112-118

Pauchard A., Alaback P.B. & Edlund E.G. (2003) Plant invasions in protected areas at multiple scales: *Linaria vulgaris* (Scrophulariaceae) in the west Yellowstone area. *Western North American Naturalist*, 63, 416-428

Pauchard A. & Shea K. (2006) Integrating the study of non-native plant invasions across spatial scales. *Biological Invasions*, 8, 399-413

PCAST (1998) Teaming with life: investing in science to understand and use America's living capita. In. PCAST (President's Committee of Advisors on Science and Technology) Panel on Biodiversity and Ecosystems

Pedroli B. & Harms B. (2001) Restoration and planning for sustainable river management: Concepts and tools - introduction. *Landscape Ecology*, 17, 1-3

Pekkarinen A. (1984) Resource partitioning and coexistence in Bumblebees (Hymenoptera, Bombinae). *Annales Entomologici Fennici*, 50, 97-107

Petanidou T. & Vokou D. (1993) Pollination ecology of Labiatae in a phryganic (East Mediterranean) ecosystem. *American Journal of Botany*, 80, 892-899

Peters R.H. (1988) Some general problems for ecology illustrated by food web theory. *Ecology*, 69, 1673-1676

Pettersson M.W. (1991) Pollination by a guild of fluctuating moth populations - option for unspecialization in *Silence vulgaris*. *Journal of Ecology*, 79, 591-604

Pickett S.T.A. (1976) Succession - evolutionary interpretation. *American Naturalist*, 110, 107-119

Pimentel D., McNair S., Janecka J., Wightman J., Simmonds C., O'Connell C., Wong E., Russel L., Zern J., Aquino T. & Tsomondo T. (2001) Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture Ecosystems & Environment*, 84, 1-20

Pimentel D., Wilson C., McCullum C., Huang R., Dwen P., Flack J., Tran Q., Saltman T. & Cliff B. (1997) Economic and environmental benefits of biodiversity. *Bioscience*, 47, 747-757

Pimentel D., Zuniga R. & Morrison D. (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*, 52, 273-288

Pimm S.L. & Lawton J.H. (1980) Are food webs divided into compartments. *Journal of Animal Ecology*, 49, 879-898

Pimm S.L., Lawton J.H. & Cohen J.E. (1991) Food web patterns and their consequences. *Nature*, 350, 669-674

Pimm S.L., Russell G.J., Gittleman J.L. & Brooks T.M. (1995) The future of biodiversity. *Science*, 269, 347-350

Planty-Tabacchi A.M., Tabacchi E., Naiman R.J., Deferrari C. & Decamps H. (1996) Invasibility of species rich communities in riparian zones. *Conservation Biology*, 10, 598-607

Potts S.G., Dafni A. & Ne'eman G. (2001) Pollination of a core flowering shrub species in Mediterranean phrygana: variation in pollinator diversity, abundance and effectiveness in response to fire. *Oikos*, 92, 71-80

Potts S.G., Vulliamy B., Dafni A., Ne'eman G. & Willmer P. (2003) Linking bees and flowers: How do floral communities structure pollinator communities? *Ecology*, 84, 2628-2642

Powell A.H. & Powell G.V.N. (1987) Population-Dynamics of male euglossine Bees in Amazonian forest fragments. *Biotropica*, 19, 176-179

Prenter J., MacNeil C., Dick J.T.A. & Dunn A.M. (2004) Roles of parasites in animal invasions. *Trends in Ecology & Evolution*, 19, 385-390

Price M.V., Waser N.M., Irwin R.E., Campbell D.R. & Brody A.K. (2005) Temporal and spatial variation in pollination of a montane herb: A seven-year study. *Ecology*, 86, 2106-2116

Price P.W. (1980) *Evolutionary biology of Parasites*. Princeton University press.

Price P.W., Westoby M., Rice B., Atsatt P.R., Fritz R.S., Thompson J.N. & Mobley K. (1986) Parasite mediation in ecological interactions. *Annual Review of Ecology and Systematics*, 17, 487-505

Prys-Jones O.E. & Corbet S.A. (1991) *Bumblebees. Naturalists' Handbooks 6*. Richmond Publishing Co. Ltd. Slough, England.

Pysek P. & Hulme P.E. (2005) Spatio-temporal dynamics of plant invasions: Linking pattern to process. *Ecoscience*, 12, 302-315

Pywell R.F., Bullock J.M., Roy D.B., Warman L.I.Z., Walker K.J. & Rothery P. (2003) Plant traits as predictors of performance in ecological restoration. *Journal of Applied Ecology*, 40, 65-77

Pywell R.F., Pakeman R.J., Allchin E.A., Bourn N.A.D., Warman E.A. & Walker K.J. (2002) The potential for lowland heath regeneration following plantation removal. *Biological Conservation*, 108, 247-258

Quince C., Higgs P.G. & McKane A.J. (2005) Deleting species from model food webs. *Oikos*, 110, 283-296

Raffaelli D. & Hall S.J. (1992) Compartments and predation in an estuarine food web. *Journal of Animal Ecology*, 61, 551-560

Raffaelli D., Van der Putten W.H., Persson L., Wardle D.A., Petchey O.L., Koricheva J., Van der Heijden M.G.A., Mikola J. & Kennedy T. (2002) Multi-trophic dynamics and ecosystem processes. In: *Biodiversity and Ecosystem Functioning* (ed. Loreau M, Naeem, S., & Inchausti, P.), pp. 147-154. Oxford University Press, Oxford

Ranta E. & Lundberg H. (1980) Resource partitioning in bumblebees - the significance of differences in proboscis length. *Oikos*, 35, 298-302

Rathcke B.J. & Jules E.S. (1993) Habitat fragmentation and plant pollinator interactions. *Current Science*, 65, 273-277

Raven P.H. (2002) Science, sustainability, and the human prospect. *Science*, 297, 1477-1477

Reay S.D. & Norton D.A. (1999) Assessing the success of restoration plantings in a temperate New Zealand forest. *Restoration Ecology*, 7, 298-308

Reid W.V. (1997) Strategies for conserving biodiversity. *Environment*, 39, 16-&

Rejmanek M. & Richardson D.M. (1996) What attributes make some plant species more invasive? *Ecology*, 77, 1655-1661

Rhoades C.C., Eckert G.E. & Coleman D.C. (1998) Effect of pasture trees on soil nitrogen and organic matter: Implications for tropical montane forest restoration. *Restoration Ecology*, 6, 262-270

Ricciardi A. (2001) Facilitative interactions among aquatic invaders: is an "invasional meltdown" occurring in the Great Lakes? *Canadian Journal of Fisheries and Aquatic Sciences*, 58, 2513-2525

Ricciardi A. & Rasmussen J.B. (1999) Extinction rates of North American freshwater fauna. *Conservation Biology*, 13, 1220-1222

Richards K.W. (1993) Non-Apis bees as crop pollinators. *Revue Suisse De Zoologie*, 100, 807-822

Richardson D.M., Allsopp N., D'Antonio C.M., Milton S.J. & Rejmanek M. (2000) Plant invasions - the role of mutualisms. *Biological Reviews*, 75, 65-93

Richardson D.M., Cowling R.M. & Lamont B.B. (1996) Non-linearities, synergisms and plant extinctions in South African fynbos and Australian kwongan. *Biodiversity and Conservation*, 5, 1035-1046

Richardson D.M., Cowling R.M. & Lemaitre D.C. (1990) Assessing the risk of invasive sSuccess in *Pinus* and *Banksia* in South-African mountain fynbos. *Journal of Vegetation Science*, 1, 629-642

Richardson D.M. & Pysek P. (2006) Plant invasions: merging the concepts of species invasiveness and community invasibility. *Progress in Physical Geography*, 30, 409-431

Robinson G.R., Quinn J.F. & Stanton M.L. (1995) Invasibility of experimental habitat islands in a California winter annual grassland. *Ecology*, 76, 786-794

Roemer G.W., Donlan C.J. & Courchamp F. (2002) Golden eagles, feral pigs, and insular carnivores: How exotic species turn native predators into prey. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 791-796

Rose R.J., Webb N.R., Clarke R.T. & Traynor C.H. (2000) Changes on the heathlands in Dorset, England, between 1987 and 1996. *Biological Conservation*, 93, 117-125

Roubik D.W. (2001) Ups and downs in pollinator populations: When is there a decline? *Conservation Ecology*, 5

Ruiz-Jaen M.C. & Aide T.M. (2005) Restoration success: How is it being measured? *Restoration Ecology*, 13, 569-577

Saber M.A., Clements D.R., Hall M.R., Doohan D.J. & Crompton C.W. (1995) The biology of Canadian weeds .105. Linaria-Vulgaris Mill. *Canadian Journal of Plant Science*, 75, 525-537

Sala O.E., Chapin F.S., Armesto J.J., Berlow E., Bloomfield J., Dirzo R., Huber-Sanwald E., Huenneke L.F., Jackson R.B., Kinzig A., Leemans R., Lodge D.M., Mooney H.A., Oesterheld M., Poff N.L., Sykes M.T., Walker B.H., Walker M. & Wall D.H. (2000) Biodiversity - Global biodiversity scenarios for the year 2100. *Science*, 287, 1770-1774

Sax D.F. & Gaines S.D. (2003) Species diversity: from global decreases to local increases. *Trends in Ecology & Evolution*, 18, 561-566

Schmid-Hempel P. (1998) *Parasites in Social Insects*. Princeton University Press. New Jersey, USA.

Schmid-Hempel P. (2000) Mating, parasites and other trials of life in social insects. *Microbes and Infection*, 2, 515-520

Schmid-Hempel R. & Muller C.B. (1991) Do parasitized bumblebees forage for their colony? *Animal Behaviour*, 41, 910-912

Schmid-Hempel P., Muller C., Schmid-Hempel R. & Shykoff J.A. (1990) Frequency and ecological correlates of parasitism by conopid flies (Conopidae, Diptera) in populations of bumblebees. *Insectes Sociaux*, 37, 14-30

Schmid-Hempel P., Puhr K., Kruger N., Reber C. & Schmid-Hempel R. (1999) Dynamic and genetic consequences of variation in horizontal transmission for a microparasitic infection. *Evolution*, 53, 426-434

Schmid-Hempel P. & Schmid-Hempel R. (1988) Parasitic flies (Conopidae, Diptera) may be important stress factors for the ergonomics of their bumblebee hosts. *Ecological Entomology*, 13, 469-472

Schmid-Hempel P. & Schmid-Hempel R. (1990) Endoparasitic larvae of conopid flies alter pollination behavior of bumblebees. *Naturwissenschaften*, 77, 450-452

Schmid-Hempel R. & Schmid-Hempel P. (1991) Endoparasitic flies, pollen collection by bumblebees and a potential host-parasite conflict. *Oecologia*, 87, 227-232

Schmid-Hempel P. & Schmid-Hempel R. (1993) Transmission of a pathogen in *Bombus terrestris*, with a note on division of labor in social insects. *Behavioral Ecology and Sociobiology*, 33, 319-327

Schmid-Hempel R. & Schmid-Hempel P. (1996) Host choice and fitness correlates for conopid flies parasitising bumblebees. *Oecologia*, 107, 71-78

Schmid-Hempel P. & Stauffer H.P. (1998) Parasites and flower choice of bumblebees. *Animal Behaviour*, 55, 819-825

Schoener T.W. (1989) Food webs from the small to the large. *Ecology*, 70, 1559-1589

Schoener T.W. & Spiller D.A. (1992) Is Extinction rate related to temporal variability in population size - an empirical answer for orb spiders. *American Naturalist*, 139, 1176-1207

Schwarz H.H. & Huck K. (1997) Phoretic mites use flowers to transfer between foraging bumblebees. *Insectes Sociaux*, 44, 303-310

SER (2004) *The SER International Primer on Ecological Restoration*. Society for Ecological Restoration International Science and Policy Working Group. Tucson, Arizona.

Shea K. & Kelly D. (1998) Estimating biocontrol agent impact with matrix models: *Carduus nutans* in New Zealand. *Ecological Applications*, 8, 824-832

Shykoff J.A. & Schmid-Hempel P. (1991a) Incidence and effects of 4 parasites in natural populations of bumble bees in Switzerland. *Apidologie*, 22, 117-125

Shykoff J.A. & Schmid-Hempel P. (1991b) Parasites delay worker reproduction in Bumblebees - consequences for eusociality. *Behavioral Ecology*, 2, 242-248

Sihag R.C. (1995) Pollination, pollinators, & pollination modes: ecological and economic importance. In: *Pollination of Cultivated Plants in the Tropics*. (ed. Roubik DW). Food and Agriculture Organisation of the United Nations Agricultural Services Bulletin 118.

Simberloff D. (1990) Reconstructing the ambiguous: can island ecosystems be restored? In: *Ecological Restoration of New Zealand islands* (eds. Towns DR, Daugherty CH & Atkinson IAE). Conservation Science publication number 2, Department of Conservation, Wellington, New Zealand

Simberloff D. (2006) Rejoinder to Simberloff (2006): Don't calculate effect sizes; Study ecological effects. *Ecology Letters*, 9, 921-922

Simberloff D. & Gibbons L. (2004) Now you see them, now you don't - population crashes of established introduced species. *Biological Invasions*, 6, 161-172

Simberloff D. & Von Holle B. (1999) Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions*, 1, 21-32

Sladen F.W.L. (1912) *The humble-bee, its life history, and how to domesticate it.* Macmillan, London, UK.

Smith K.G.V. (1969) *Handbooks for the Identification of British Insects: Volume X. Part 3 (a) Diptera Conopidae*. Royal Entomological Society of London.

Smyth J.D. (1994) *Introduction to animal parsitiology*. Cambridge University Press, UK.

Sokal R.R. & Rohlf F.J. (1995) *Biometry*. Freeman & Co, New York.

Sole R.V. & Montoya J.M. (2001) Complexity and fragility in ecological networks. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 268, 2039-2045

Sole R.V. & Montoya J.M. (2006) Ecological network meltdown from habitat loss and fragmentation. In: *Ecological networks: linking structure to dynamics in food webs* (eds. Pascual M & Dunne JA), pp. 305-351. Oxford University Press

Southwood T.R.E. (1977) Habitat, templet for ecological strategies – presidential address to British Ecological Society, 5 January 1977. *Journal of Animal Ecology*, 46, 337-365

Spellerberg I.F. (1989) An assessment of the importance of heathlands as habitats for reptiles. *Botanical Journal of the Linnean Society*, 101, 313-318

Spira T.P. (2001) Plant-pollinator interactions: A threatened mutualism with implications for the ecology and management of rare plants. *Natural Areas Journal*, 21, 78-88

Stachon W.J. & Zimdahl R.L. (1980) Allelopathic Activity of Canada Thistle (Cirsium-Arvense) in Colorado. *Weed Science*, 28, 83-86

Stachowicz J.J. (2001) Mutualism, facilitation, and the structure of ecological communities. *Bioscience*, 51, 235-246

Stanton M., Young H.J., Ellstrand N.C. & Clegg J.M. (1991) Consequences of floral variation for male and female reproduction in experimental populations of Wild Radish, *Raphanus sativus* L. *Evolution*, 45, 268-280

Steffan-Dewenter I. & Tscharntke T. (1999) Effects of habitat isolation on pollinator communities and seed set. *Oecologia*, 121, 432-440

Steffan-Dewenter I. (2002) Landscape context affects trap-nesting bees, wasps, and their natural enemies. *Ecological Entomology*, 27, 631-637

Steffan-Dewenter I. & Tscharntke T. (2000) Resource overlap and possible competition between honey bees and wild bees in central Europe. *Oecologia*, 122, 288-296

Stohlgren T.J., Binkley D., Chong G.W., Kalkhan M.A., Schell L.D., Bull K.A., Otsuki Y., Newman G., Bashkin M. & Son Y. (1999) Exotic plant species invade hot spots of native plant diversity. *Ecological Monographs*, 69, 25-46

Stout J.C., Allen J.A. & Goulson D. (2000) Nectar robbing, forager efficiency and seed set: Bumblebees foraging on the self incompatible plant *Linaria vulgaris* (Scrophulariaceae). *Acta Oecologica-International Journal of Ecology*, 21, 277-283

Suding K.N., Gross K.L. & Houseman G.R. (2004) Alternative states and positive feedbacks in restoration ecology. *Trends in Ecology & Evolution*, 19, 46-53

Swift J. (1733) *On Poetry, A Rhapsody.*

Symes N.C. & Day J. (2003) *A practical guide to the restoration and management of lowland heathland.* The RSPB, Sandy, U.K.

Taraschewski H. (2006) Hosts and parasites as aliens. *Journal of Helminthology*, 80, 99-128

Tepedino V.J. (1981) The pollination efficiency of the squash bee (*Peponapis pruinosa*) and the Honey Bee (*Apis mellifera*) on Summer Squash (*Cucurbita pepo*). *Journal of the Kansas Entomological Society*, 54, 359-377

Thompson H.M. (2001) Assessing the exposure and toxicity of pesticides to bumblebees (*Bombus* spp.). *Apidologie*, 32, 305-321

Thomson J.D. (1981) Spatial and temporal components of resource assessment by flower-feeding insects. *Journal of Animal Ecology*, 50, 49-59

Thompson J.N. & Pellmyr O. (1992) Mutualism with pollinating seed parasites amid co-pollinators - constraints on specialization. *Ecology*, 73, 1780-1791

Thompson J.N., Reichman O.J., Morin P.J., Polis G.A., Power M.E., Sterner R.W., Couch C.A., Gough L., Holt R., Hooper D.U., Keesing F., Lovell C.R., Milne B.T., Molles M.C., Roberts D.W. & Strauss S.Y. (2001) Frontiers of ecology. *Bioscience*, 51, 15-24

Thuiller W., Richardson D.M., Rouget M., Proches S. & Wilson J.R.U. (2006) Interactions between environment, species traits, and human uses describe patterns of plant invasions. *Ecology*, 87, 1755-1769

Tilman D. (1999) The ecological consequences of changes in biodiversity: A search for general principles. *Ecology*, 80, 1455-1474

Tilman D. (2004) Niche tradeoffs, neutrality, and community structure: A stochastic theory of resource competition, invasion, and community assembly. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 10854-10861

Tilman D., Wedin D. & Knops J. (1996) Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature*, 379, 718-720

Toole E.H. & Brown E. (1946) Final results of the Duval buried seed experiment. *Journal of Agricultural Research*, 72, 201-210

Traveset A. & Richardson D.M. (2006) Biological invasions as disruptors of plant reproductive mutualisms. *Trends in Ecology & Evolution*, 21, 208-216

Tscharntke T. & Brandl R. (2004) Plant-insect interactions in fragmented landscapes. *Annual Review of Entomology*, 49, 405-430

Tscharntke T., Gathmann A. & Steffan-Dewenter I. (1998) Bioindication using trap-nesting bees and wasps and their natural enemies: community structure and interactions. *Journal of Applied Ecology*, 35, 708-719

Tylianakis J.M., Tscharntke T. & Lewis O.T. (2007) Habitat modification alters the structure of tropical host-parasitoid food webs. 445, 202-205

Valtonen E.T., Holmes J.C., Aronen J. & Rautalahti I. (2003) Parasite communities as indicators of recovery from pollution: parasites of roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*) in Central Finland. *Parasitology*, 126, S43-S52

van Dorp D., Schippers P. & van Groenendael J.M. (1997) Migration rates of grassland plants along corridors in fragmented landscapes assessed with a cellular automation model. *Landscape Ecology*, 12, 39-50

van Wilgen B.W., Cowling R.M. & Burgers C.J. (1996) Valuation of ecosystem services. *Bioscience*, 46, 184-189

Vander Zanden M.J., Olden J.D. & Zedler J.B. (2006) Food-web approaches in restoration ecology. In: *Foundations of restoration ecology* (eds. Falk DA, Palmer MA & Zedler L), pp. 165-189. Island Press

Vazquez D.P., Morris W.F. & Jordano P. (2005) Interaction frequency as a surrogate for the total effect of animal mutualists on plants *Ecology Letters*, 8, 1088-1094

Vazquez D.P., Poulin R., Krasnov B.R. & Shenbrot G.I. (2005) Species abundance and the distribution of specialization in host-parasite interaction networks. *Journal of Animal Ecology*, 74, 946-955

Vermeij G.J. (1996) An agenda for invasion biology. *Biological Conservation*, 78, 3-9

Vitousek P.M. (1990) Biological invasions and ecosystem processes - towards an integration of population biology and ecosystem studies. *Oikos*, 57, 7-13

Vitousek P.M., D'Antonio C.M., Loope L.L. & Westbrooks R. (1996) Biological invasions as global environmental change. *American Scientist*, 84, 468-478

Vitousek P.M., Mooney H.A., Lubchenco J. & Melillo J.M. (1997a) Human domination of Earth's ecosystems. *Science*, 277, 494-499

Vitousek P.M., D'Antonio C.M., Loope L.L., Rejmanek M. & Westbrooks R. (1997b) Introduced species: A significant component of human-caused global change. *New Zealand Journal of Ecology*, 21, 1-16

Vitousek P.M., Walker L.R., Whiteaker L.D., Muellerdombois D. & Matson P.A. (1987) Biological Invasion by *Myrica faya* Alters Ecosystem Development in Hawaii. *Science*, 238, 802-804

Von Holle B., Joseph K.A., Largay E.F. & Lohnes R.G. (2006) Facilitations between the introduced nitrogen-fixing tree, *Robinia pseudoacacia*, and nonnative plant species in the glacial outwash upland ecosystem of Cape Cod, MA. *Biodiversity and Conservation*, 15, 2197-2215

Walker B.H. (1992) Biodiversity and ecological redundancy. *Conservation Biology*, 6, 18-23

Walker K.J., Pywell R.F., Warman E.A., Fowbert J.A., Bhogal A. & Chambers B.J. (2004) The importance of former land use in determining successful re-creation of lowland heath in southern England. *Biological Conservation*, 116, 289-303

Walters B.B. (2000) Local mangrove planting in the Philippines: Are fisherfolk and fishpond owners effective restorationists? *Restoration Ecology*, 8, 237-246

Waser N.M., Chittka L., Price M.V., Williams N.M. & Ollerton J. (1996) Generalization in pollination systems, and why it matters. *Ecology*, 77, 1043-1060

Waser N.M. & Ollerton J. (2006) *Plant-pollinator interactions: from specialisation to generalization.* The University of Chicago Press, Chicago, USA.

Webb N.R. & Haskins L.E. (1980) An ecological survey of heathlands in the Poole Basin, Dorset, England, in 1978. *Biological Conservation*, 17, 281-296

Westphal C., Steffan-Dewenter I. & Tscharntke T. (2003) Mass flowering crops enhance pollinator densities at a landscape scale. *Ecology Letters*, 6, 961-965

White P.S. & Walker J.L. (1997) Approximating nature's variation: Selecting and using reference information in restoration ecology. *Restoration Ecology*, 5, 338-349

White E.M., Wilson J.C. & Clarke A.R. (2006) Biotic indirect effects: a neglected concept in invasion biology. *Diversity and Distributions*, 12, 443-455

Wilcove D.S., Rothstein D., Dubow J., Phillips A. & Losos E. (1998) Quantifying threats to imperiled species in the United States. *Bioscience*, 48, 607-615

Wilkins S., Keith D.A. & Adam P. (2003) Measuring success: Evaluating the restoration of a grassy eucalypt woodland on the Cumberland Plain, Sydney, Australia. *Restoration Ecology*, 11, 489-503

Williams C.S. (1995) Conserving Europe bees - Why all the buzz. *Trends in Ecology & Evolution*, 10, 309-310

Williams N.M., Minckley R.L. & Silveira F.A. (2001) Variation in native bee faunas and its implications for detecting community changes. *Conservation Ecology*, 5

Williams P.H. (1982) The distribution and decline of British bumble bees (*Bombus* Latr). *Journal of Apicultural Research*, 21, 236-245

Williams P.H. (1986) Environmental change and the distributions of British bumble bees (*Bombus* Latr). *Bee World*, 67, 50-61

Williams P.H. (1988) Habitat use by bumble bBees (*Bombus* Spp). *Ecological Entomology*, 13, 223-237

Williams P.H. (2005) Does specialization explain rarity and decline British bumblebees? - A response to Goulson et al. *Biological Conservation*, 122, 33-43

Williamson M.H. & Fitter A. (1996) The characters of successful invaders. *Biological Conservation*, 78, 163-170

Willmer P.G., Bataw A.A.M. & Hughes J.P. (1994) The superiority of Bumblebee to honeybees as pollinators - Insect Visits to Raspberry Flowers. *Ecological Entomology*, 19, 271-284

Wilson E.O.W. (1992) *The Diversity of Life.* W. Norton & Company, New York, NY.

Wilson P. & Thomson J.D. (1991) Heterogeneity among floral visitors leads to Discordance between removal and deposition of pollen. *Ecology*, 72, 1503-1507

Winter, K., Adams, L., Thorp, R., Inouye, D., Day, L., Ascher, J., Buchmann, S. (2006) *Importation of non-native bumble bees into North America: Potential consequences of using Bombus terrestris and other non-native bumble bees for greenhouse crop pollination in Canada, Mexico and the United States.* A White Paper of the North American Pollinator Protection Campaign (NAPPC)

Wiser S.K., Allen R.B., Clinton P.W. & Platt K.H. (1998) Community structure and forest invasion by an exotic herb over 23 years. *Ecology*, 79, 2071-2081

Xu H.G., Ding H., Li M.Y., Qiang S., Guo J.Y., Han Z.M., Huang Z.G., Sun H.Y., He S.P., Wu H.R. & Wan F.H. (2006) The distribution and economic losses of alien species invasion to China. *Biological Invasions*, 8, 1495-1500

Yodzis P. (1980) The connectance of real ecosystems. *Nature*, 284, 544-545

Young T.P. (2000) Restoration ecology and conservation biology. *Biological Conservation*, 92, 73-83

Young T.P., Petersen D.A. & Clary J.J. (2005) The ecology of restoration: historical links, emerging issues and unexplored realms. *Ecology Letters*, 8, 662-673

Zanden M.J.V., Olden J.D. & Gratton C. (2006) Food-Web Approaches in Restoration Ecology. In: *Foundations of Restoration Ecology* (eds. Falk DA, Palmer MA & Zedler J). Island Press

Zavaleta E.S., Hobbs R.J. & Mooney H.A. (2001) Viewing invasive species removal in a whole-ecosystem context. *Trends in Ecology & Evolution*, 16, 454-459

Zedler J.B. (1993) Canopy Architecture of Natural and Planted Cordgrass Marshes - Selecting Habitat Evaluation Criteria. *Ecological Applications*, 3, 123-138

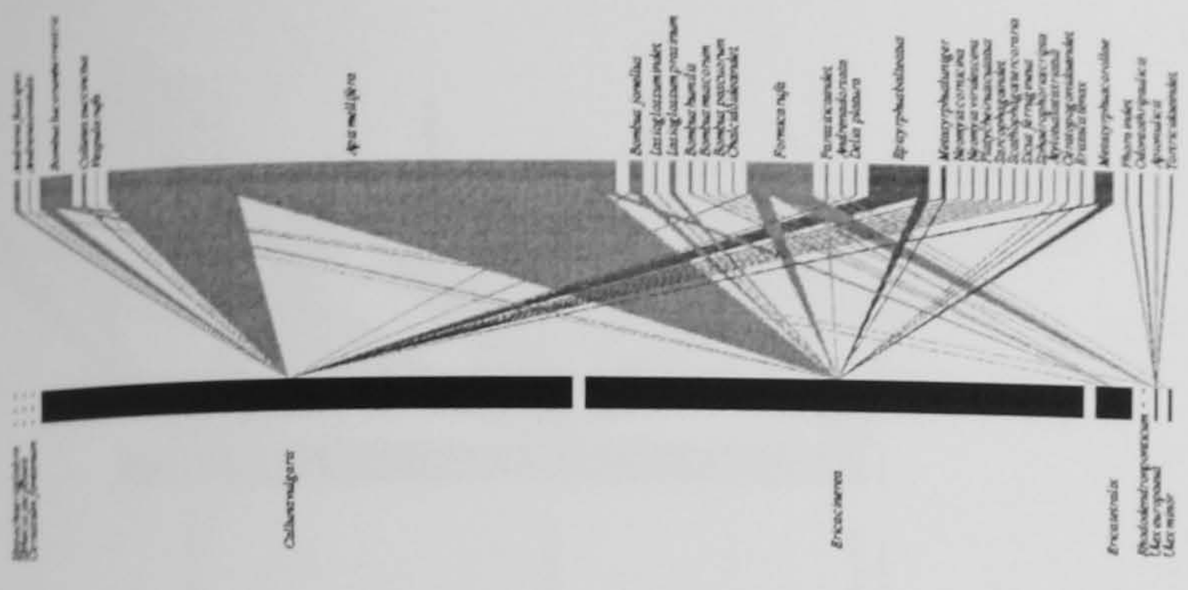
Zedler J.B. & Callaway J.C. (1999) Tracking wetland restoration: Do mitigation sites follow desired trajectories? *Restoration Ecology*, 7, 69-73

APPENDICES

Appendix A

a)Ancient Heaths (2001)

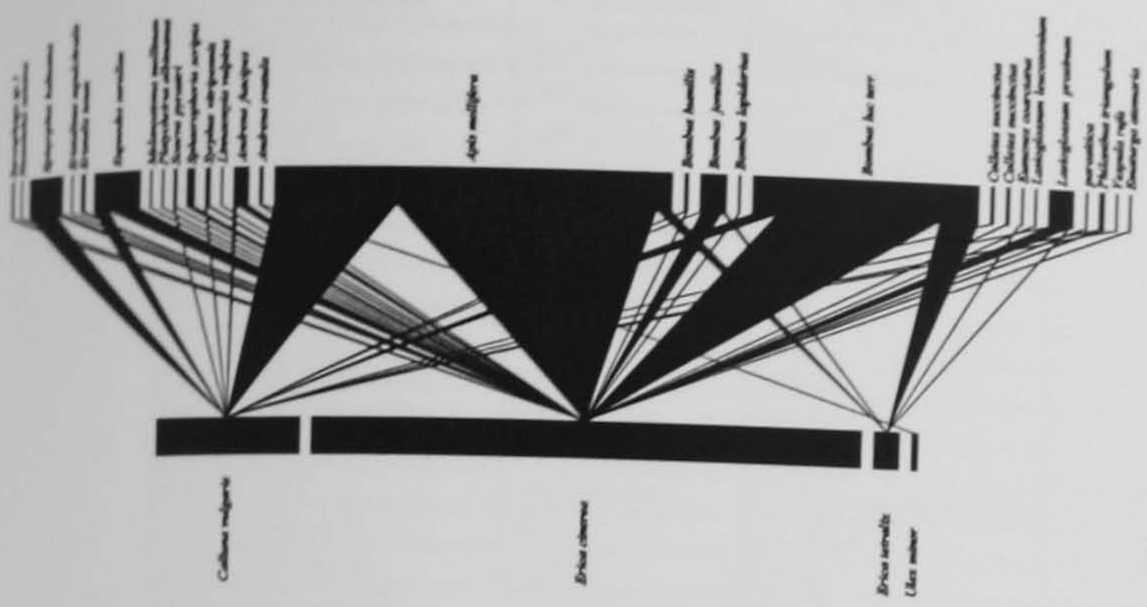
b)Restored Heaths (2001)

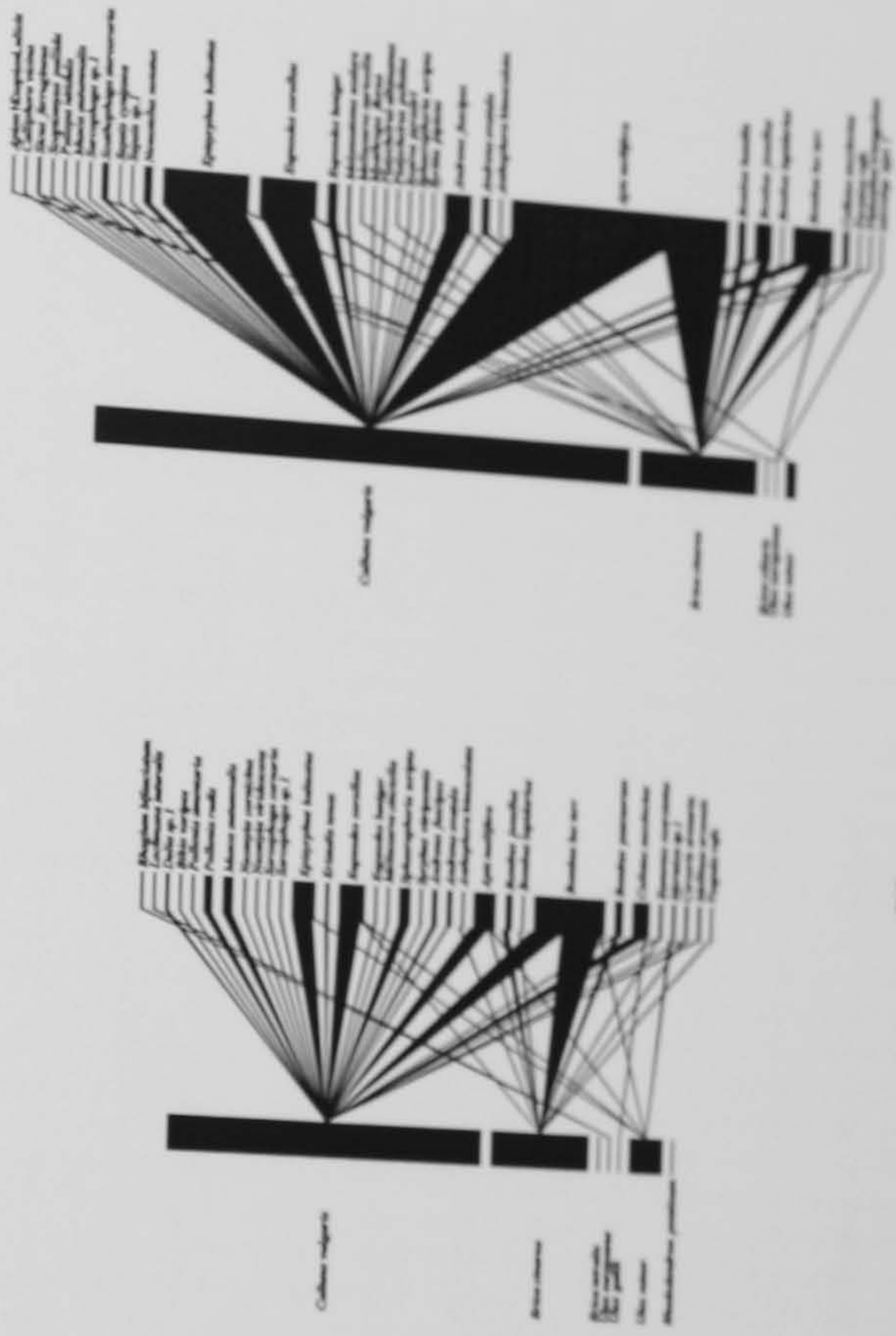


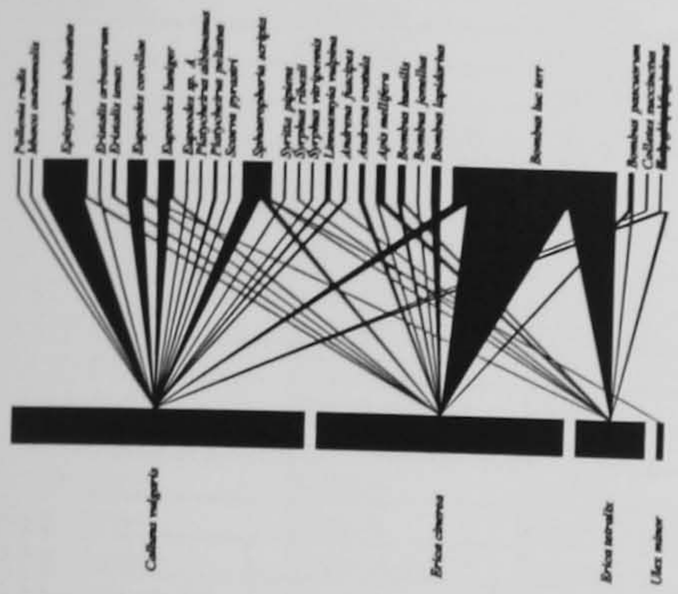
A.1 Quantitative interaction networks representing interactions between the heathland plant community and their insect visitors in 2001 by M.L. Forup. Networks are of a) ancient and b) restored heathland at Arne (Pair 1), Gore (Pair 2), Hyde (Pair 3), and Morden (Pair 4). The bottom row of rectangular bars represents the flower species whilst the upper row represents the insect visitors. Each rectangular bar in the rows represents a single species, their relative abundance being proportional to bar width. The scale bar at the base of the figure represents the number of floral units per m^2 of heathland and the number of visiting insects. The solid triangles that connect the two trophic levels represent the interactions linking the plant and insect species, the size of the triangle being proportional to the frequency of the interaction. All eight webs are drawn to the same scale.

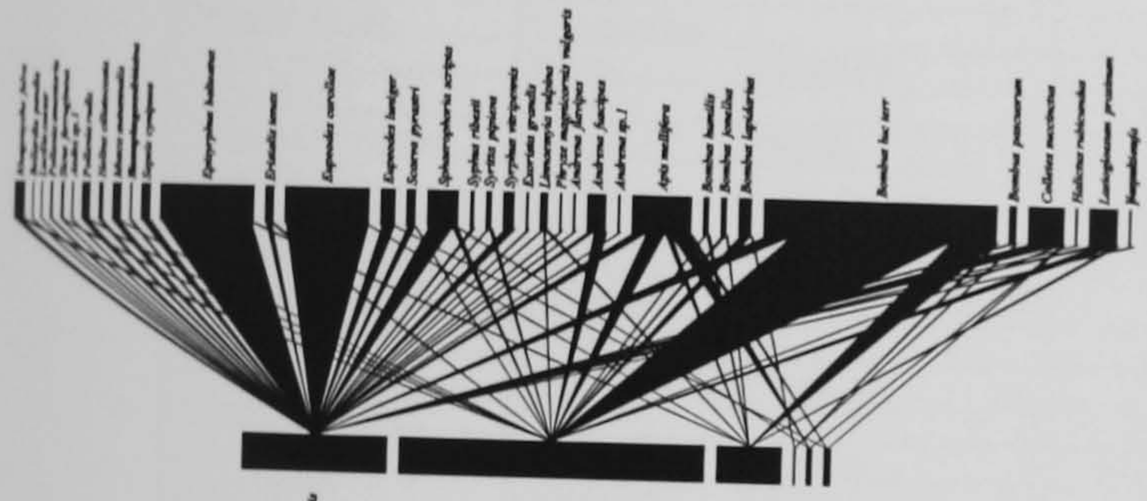
a)Ancient Heaths (2004)

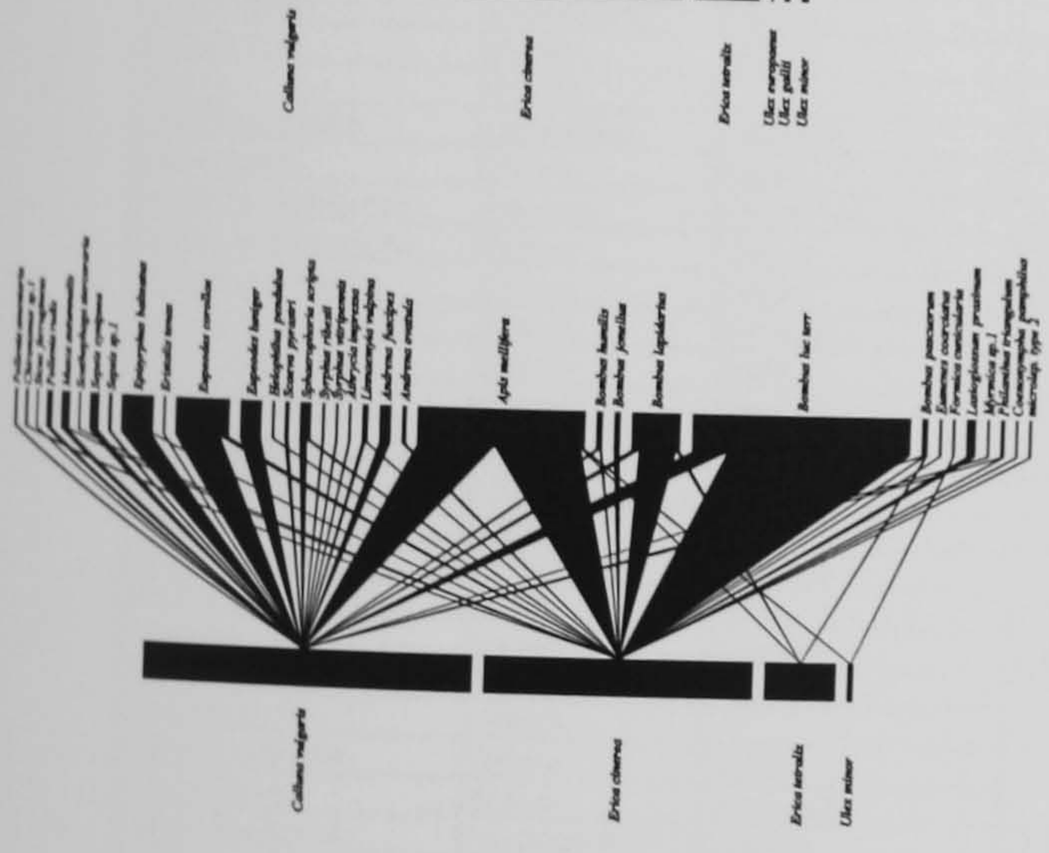
b)Restored Heaths (2004)

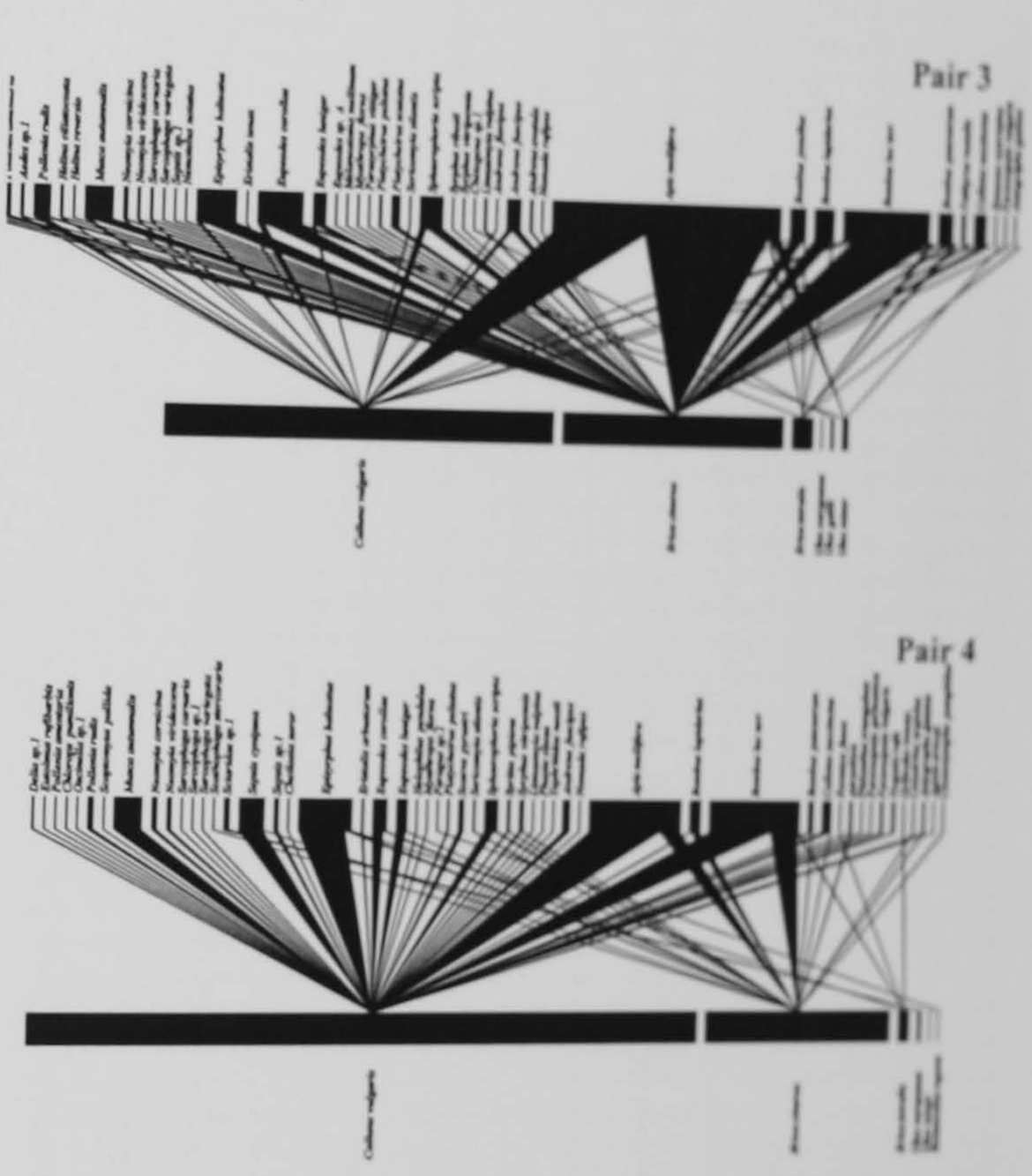












100 Flowers

100 Insect visitors

A.2 Quantitative interaction networks representing interactions between the heathland plant community and their insect visitors in 2004 by K.S.E. Henson. Networks are for a) ancient and b) restored heathland at Arne (Pair 1), Gore (Pair 2), Hyde (Pair 3), and Morden (Pair 4). The bottom row of rectangular bars represents the flower species whilst the upper row represents the insect visitors. Each rectangular bar in the rows represents a single species, their relative abundance being proportional to bar width. The scale bar at the base of the figure represents the number of floral units per m^2 of heathland and the number of visiting insects. The solid triangles that connect the two trophic levels represent the interactions linking the plant and insect species, the size of the triangle being proportional to the frequency of the interaction. All eight webs are drawn to the same scale.

			Restored heathland								Ancient heathland							
			2001				2004				2001				2004			
Insect Species	Insect Order	Authority	Pair 1	Pair 2	Pair 3	Pair 4	Pair 1	Pair 2	Pair 3	Pair 4	Pair 1	Pair 2	Pair 3	Pair 4	Pair 1	Pair 2	Pair 3	Pair 4
Aedes caspius	Diptera	Pallus	•															
Aedes sp.1	Diptera							•				•						
Aglais urticae	Lepidoptera	Linnaeus				•				•								
Andrena dorsata	Hymenoptera	Kirby			•											•	•	
Andrena flavipes	Hymenoptera	Panzer			•			•				•					•	
Andrena fuscipes	Hymenoptera	Kirby	•	•			•	•	•	•	•	•	•	•	•	•	•	•
Andrena ovatula	Hymenoptera	Kirby					•	•			•		•	•	•	•	•	•
Andrena sp.1	Hymenoptera											•						
Anthophora bimaculata	Hymenoptera	Panzer					•		•									
Apion (Exapion) ulicis	Coleoptera	Forster	•	•	•		•									•	•	•
Apis mellifera	Hymenoptera	Linnaeus	•	•	•	•	•	•	•	•	•	•	•	•	•	•		•
Aricia agestis	Lepidoptera	Denis & Schiffermüller																
Athrycia impressa	Diptera	v.d. Wulp												•				
Atylotus latistriatus	Diptera	Brauer													•			
Autographa gamma	Lepidoptera	Linnaeus			•			•		•								
Bellardia pandia	Diptera	Walker										•						
Bellardia viarum	Diptera	Robineau-Desvoidy		•													•	
Bibio nigriventris	Diptera	Haliday	•		•													
Bibio varipes	Diptera	Meigen							•									
Bicellaria pilosa	Diptera	Lundbeck			•													
Bombus hortorum	Hymenoptera	Linnaeus															•	
Bombus humilis	Hymenoptera	Illiger	•	•	•		•				•	•	•	•	•	•	•	•
Bombus jonellus	Hymenoptera	Kirby	•	•	•	•	•	•	•		•	•	•	•	•	•	•	•
Bombus lapidarius	Hymenoptera	Linnaeus		•	•	•	•	•	•	•	•	•	•	•		•	•	•
Bombus luc/terr	Hymenoptera	Linnaeus	•	•	•	•				•	•	•			•		•	•
Bombus lucorum	Hymenoptera	Linnaeus					•	•	•	•	•	•	•	•		•		•
Bombus muscorum	Hymenoptera	Linnaeus		•	•										•			
Bombus pascuorum	Hymenoptera	Scopoli		•		•		•	•	•		•	•	•	•	•	•	•
Bombus terrestris	Hymenoptera	Linnaeus					•	•	•	•	•	•	•	•				
Brachicoma devia	Diptera	Fallen			•												•	
Calliphora vicina	Diptera	Robineau-Desvoidy	•			•	•											
Celastrina argiolus	Lepidoptera	Linnaeus								•								
Ceratopogonidae indet.	Diptera														•			
Cerceris arenaria	Hymenoptera	Linnaeus			•	•			•				•					
Ceridomyiidae indet.	Diptera					•												
Chalcidoidea indet.	Hymenoptera														•			
Cheilosia soror	Diptera	Zetterstedy								•								
Chetogena sp.1	Diptera							•										
Chironomus annularius	Diptera	Degeer				•												
Chironomus sp.1	Diptera													•				
Chlorops pumilionis	Diptera	Bjerkander								•								
Coccinella 7-punctata	Coleoptera	Linnaeus		•	•													
Coenonympha pamphilus	Lepidoptera	Linnaeus								•				•				
Coenosia indet.	Diptera																•	
Coenosia tigrina	Diptera	Fabricius															•	•
Coleoptera indet. 1	Coleoptera			•													•	
Colletes succinctus	Hymenoptera	Linnaeus	•		•	•	•	•	•	•	•	•	•		•	•	•	•
Cynthia cardui	Lepidoptera	Linnaeus															•	
Delia platura	Diptera	Meigen						•							•	•		
Delia sp.1	Diptera							•	•	•								
Ematurga atomaria	Lepidoptera	Linnaeus									•							
Empis praevia	Diptera	Collin															•	
Enallagma cyathigerum	Odonata	Charpentier				•												
Epistrophe grossulariae	Diptera	Meigen	•	•	•	•											•	•
Episyrphus balteatus	Diptera	Degeer	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Eristalinus sepulchralis	Diptera	Linnaeus									•							
Eristalis abusivus	Diptera	Collin				•												
Eristalis arbustorum	Diptera	Linnaeus			•					•			•					
Eristalis pertinax	Diptera	Scopoli	•															
Eristalis tenax	Diptera	Linnaeus		•		•		•	•		•	•	•	•	•	•	•	•
Eudasyphora cyanella	Diptera	Meigen															•	
Eumenes coarctatus	Hymenoptera	Linnaeus						•	•		•			•				
Eumerus strigatus	Diptera	Fallen															•	•
Eupeodes corollae	Diptera	Fabricius					•	•	•	•	•	•	•	•				
Eupeodes luniger	Diptera	Meigen					•	•	•	•		•	•	•				
Eupeodes sp. A	Diptera							•					•					
Eutolmus rufibarbis	Diptera	Meigen								•		•						
Exorista grandis	Diptera	Zetterstedt												•				
Formica cunicularia	Hymenoptera	Latrieille															•	•
Formica fusca	Hymenoptera	Linnaeus		•	•	•									•			
Formica rufa	Hymenoptera	Linnaeus	•	•			•			•								
Graphomya picta	Diptera	Zetterstedt		•								•						
Halictus rubicundus	Hymenoptera	Christ															•	
Hebecnema umbratica	Diptera	Meigen						•										
Helina calceata	Diptera	Rondani										•						
Helina ciliatocosta	Diptera	Zetterstedt						•										
Helina reversio	Diptera	Harris		•						•				•	•	•	•	
Helophilus pendulus	Diptera	Linnaeus	•	•	•	•											•	
Hydrellia griseola	Diptera	Fallen															•	
Hydrophoria lancifer	Diptera	Harris		•												•		
Hydrotaea irritans	Diptera	Fallen																•
Inachis io	Lepidoptera	Linnaeus																
Ischnorhynchus geminatus	Hemiptera	Fieber		•											•			
Lassioglossum caceatum	Hymenoptera	Scopoli																
Lasioglossum indet.	Hymenoptera																	

A.3 Presence/absence table of the insect species sampled from four pairs of restored and ancient dry, lowland heathlands located in south Dorset, England. Sampling was carried out in 2001 by M.L. Forup and in 2004 by KS.E. Henson.

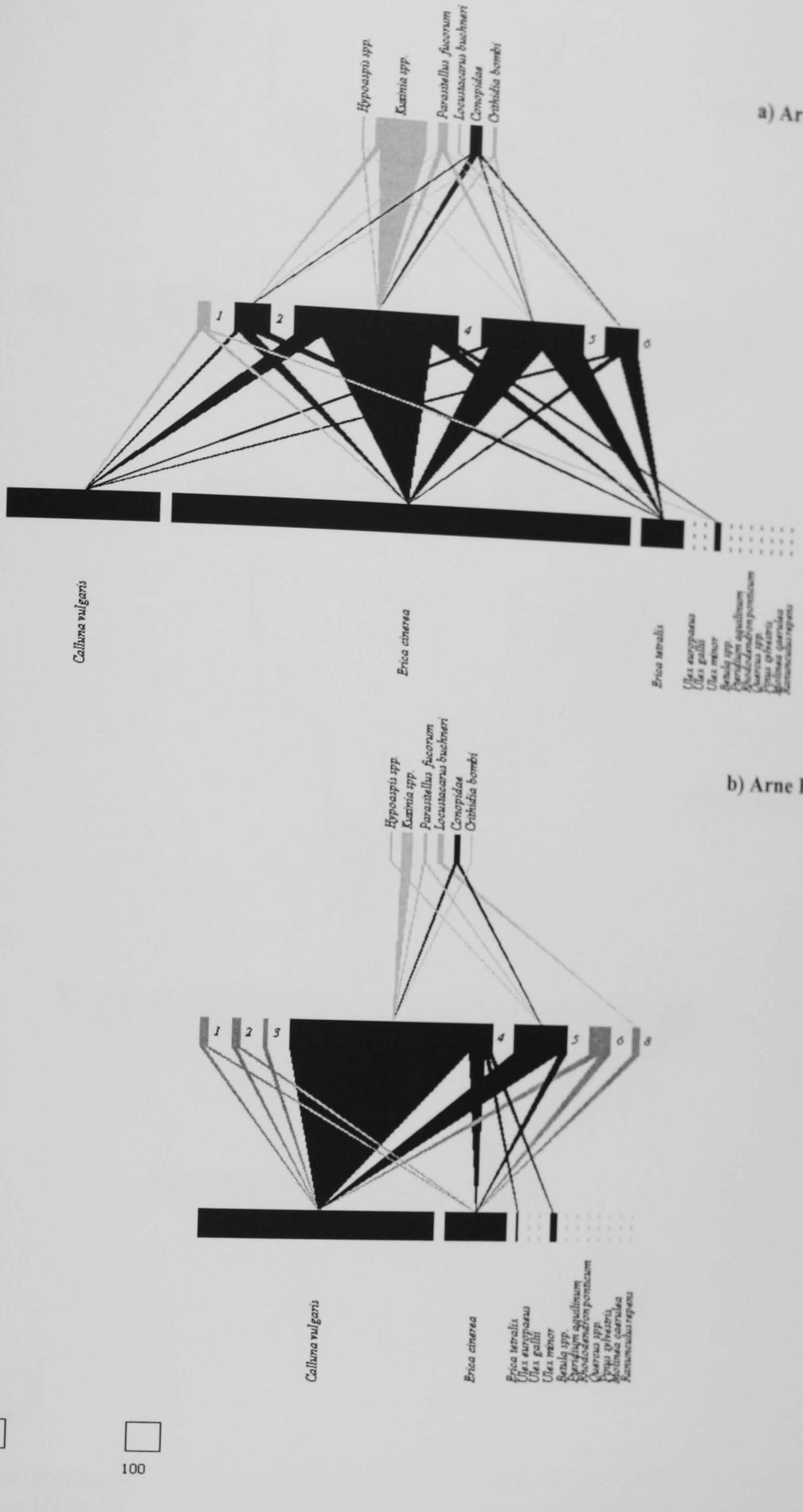
			Restored heathland								Ancient heathland							
			2001				2004				2001				2004			
Insect Species	Insect Order	Authority	Pair 1	Pair 2	Pair 3	Pair 4	Pair 1	Pair 2	Pair 3	Pair 4	Pair 1	Pair 2	Pair 3	Pair 4	Pair 1	Pair 2	Pair 3	Pair 4
Lasioglossum lativentre	Hymenoptera	Schenk																
Lasioglossum leucozonium	Hymenoptera	Schrank																
Lasioglossum prasinum	Hymenoptera	Smith									•						•	
Lasius niger	Hymenoptera	Linnaeus									•	•		•	•	•		
Linnaemyia vulpina	Diptera	Fabricius		•	•	•		•		•	•	•	•	•			•	
Lochmaea suturalis	Coleoptera	Thomson							•							•	•	•
Lucilia caesar	Diptera	Linnaeus										•						
Lucilia richardsi	Diptera	Collin																•
Lucilia sericata	Diptera	Meigen														•		
Machimus atricapillus	Diptera	Fallen				•										•		
Melanargia galathea	Lepidoptera	Linnaeus			•													
Melanostoma mellinum	Diptera	Linnaeus						•			•							
Melanostoma scalare	Diptera	Fabricius					•											
Meligramma euchroma	Diptera	Kowarz					•											
Melinda gentilis	Diptera	Robineau-Desvoidy				•												
Meliscaeva cinctella	Diptera	Zetterstedt							•									
Mellinus arvensis	Hymenoptera	Linnaeus							•									
Metasyrphus corollae	Diptera	Fabricius	•	•	•	•												
Metasyrphus latifasciatus	Diptera	Macquart													•	•	•	•
Metasyrphus luniger	Diptera	Meigen		•	•											•		
microlep. type 1	Lepidoptera						•								•	•	•	
microlep. type 2	Lepidoptera																	
Musca autumnalis	Diptera	Degeer	•				•	•	•	•		•	•	•				
Myathropa florae	Diptera	Linnaeus				•	•	•		•							•	•
Myopa fasciata	Diptera	Meigen												•				
Myrmica sp.1	Hymenoptera								•							•		
Nemotelus notatus	Diptera	Zetterstedt					•	•			•							
Neomyia cornicina	Diptera	Fabricius		•				•	•	•		•			•			
Neomyia viridescens	Diptera	Robineau-Desvoidy						•	•	•					•			
Nomada rufipes	Hymenoptera	Fabricius						•		•					•			
Ochlodes venata	Lepidoptera	Bremer & Grey								•								
Odontothrips ulicis	Thysanoptera	Haliday																
Orthocladiinae indet.	Diptera				•										•			
Oscinella sp.1	Diptera									•								
Paragus sp.1	Diptera									•								
parasitica	Hymenoptera				•					•	•	•			•	•	•	•
Parasyrphus vittiger	Diptera	Zetterstedt						•										
Paravespula germanica	Hymenoptera	Fabricius								•								
Paravespula vulgaris	Hymenoptera	Linnaeus						•		•								
Phasia obesa	Diptera	Fabricius								•								
Philanthus triangulum	Hymenoptera	Fabricius	•				•			•	•			•		•		•
Phora indet.	Diptera														•			
Phryxe magnicornis	Diptera	Zetterstedt										•						
Platycheirus albimanus	Diptera	Fabricius		•	•		•				•		•			•	•	•
Platycheirus peltatus	Diptera	Meigen					•	•		•			•					
Platycheirus scutatus	Diptera	Meigen				•		•							•			
Pollenia amentaria	Diptera	Scopoli			•			•	•	•		•		•		•		
Pollenia angustigena	Diptera	Wainwright			•												•	
Pollenia rudis	Diptera	Fabricius		•				•	•	•		•	•	•		•		
Pollenia viatica	Diptera	Robineau-Desvoidy		•	•												•	
Polydrusus confluens	Coleoptera	Stephens		•	•													
Polyommatus icarus	Lepidoptera	Rottemburg											•					
Psilopa nitidula	Diptera	Fallen					•											
Psithyrus vestalis	Hymenoptera	Geoffroy						•										•
Rhagio tringaria	Diptera	Linnaeus															•	
Rhagium bifasciatum	Coleoptera	Fabricius							•									
Rhagonycha fulva	Coleoptera	Scopoli	•	•	•							•				•	•	•
Rhingia campestris	Diptera	Meigen														•		
Sarcophaga carnaria	Diptera	Linnaeus			•			•	•	•						•	•	
Sarcophaga incisilobata	Diptera	Pandellé														•		
Sarcophaga sp.1	Diptera			•	•	•	•		•	•	•				•	•	•	•
Sarcophaga subvicina	Diptera	Rhodendorf										•					•	
Sarcophaga variegata	Diptera	Scopoli			•			•		•						•		
Scaeva pyrastri	Diptera	Linnaeus					•			•	•	•	•	•				
Scaptomyza pallida	Diptera	Zetterstedt					•			•								
Scathophaga stercoraria	Diptera	Linnaeus	•		•		•			•					•	•		
Sciaridae sp.1	Diptera									•		•		•				
Sepsis cynipsea	Diptera	Linnaeus					•			•							•	
Sepsis sp.1	Diptera					•	•	•		•								
Sericomyia silentis	Diptera	Harris	•	•	•	•		•		•							•	
Sericus brunneus	Coleoptera	Linnaeus										•		•	•	•		•
Sicus ferrugineus	Diptera	Linnaeus				•	•											•
Siphona urbana	Diptera	Harris																
Sphaerophoria menthastri	Diptera	Linnaeus *sensu* Vockeroth		•													•	
Sphaerophoria philanthus	Diptera	Meigen		•														
Sphaerophoria scripta	Diptera	Linnaeus	•		•	•	•	•	•	•	•	•	•	•			•	•
Syritta pipiens	Diptera	Linnaeus	•				•			•		•	•					
Syrphus ribesii	Diptera	Linnaeus						•				•	•	•				
Syrphus vitripennis	Diptera	Meigen						•	•	•	•	•	•					•
Tachina grossa	Diptera	Linnaeus			•					•								
Tephritidae neesii	Diptera	Meigen													•			
Tortricidae indet.	Lepidoptera																	
Trypetoptera punctulata	Diptera	Scopolo			•											•	•	
Vespula rufa	Hymenoptera	Linnaeus		•					•	•	•	•	•					

A.3 (continued)

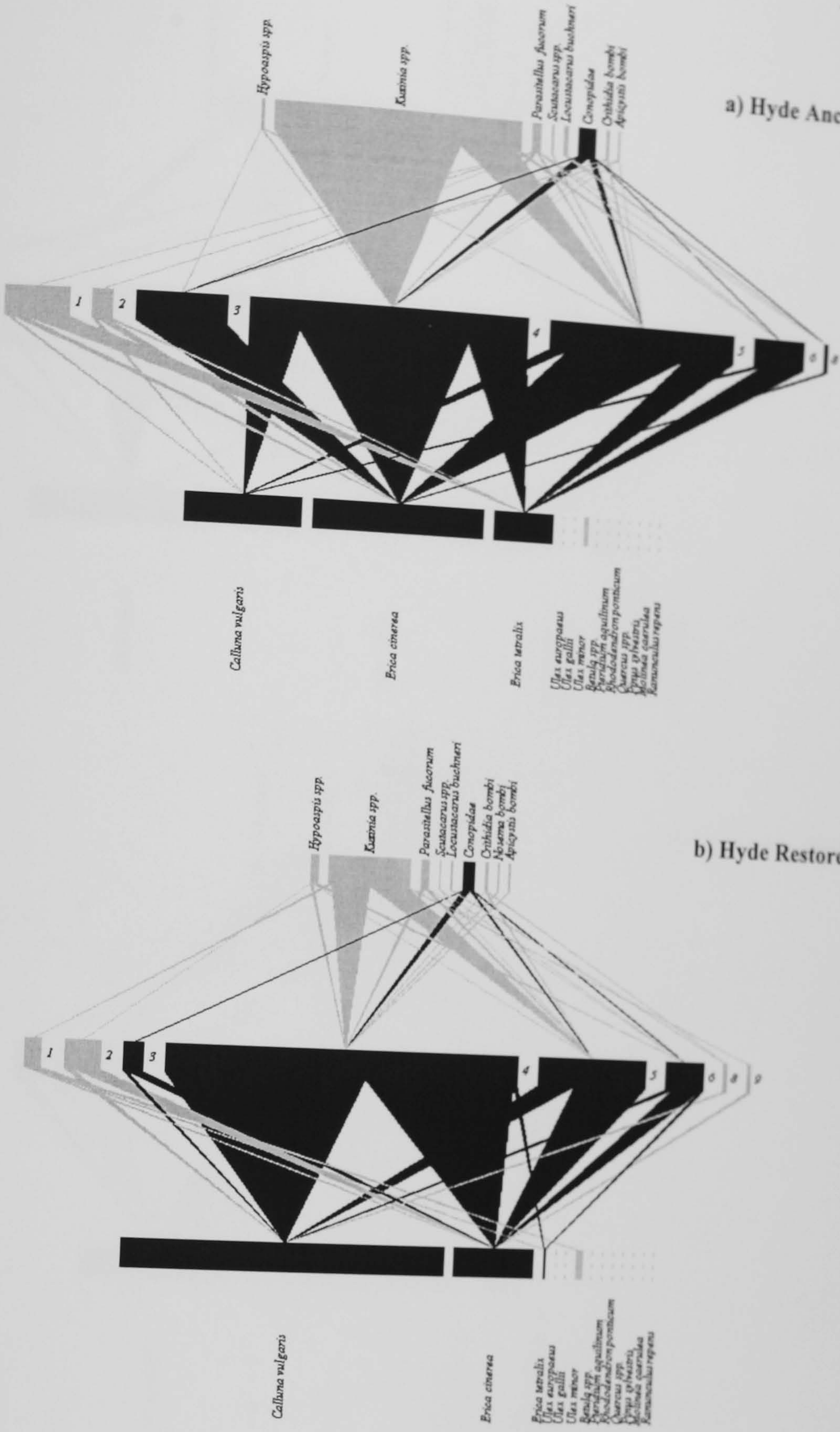
Heathland Flora: 1) *Calluna vulgaris,* 2) *Erica tetralix,*3) *Erica cinerea,* 4) *Ulex minor, Erica ciliaris,* 5) *Ulex Gallii,* 6)*Erica ciliaris*

Dry, lowland heathland in south Dorset: 1) Godlingston heath, 2) Grip heath at Arne, & 3) Great Ovens heath, Gore.

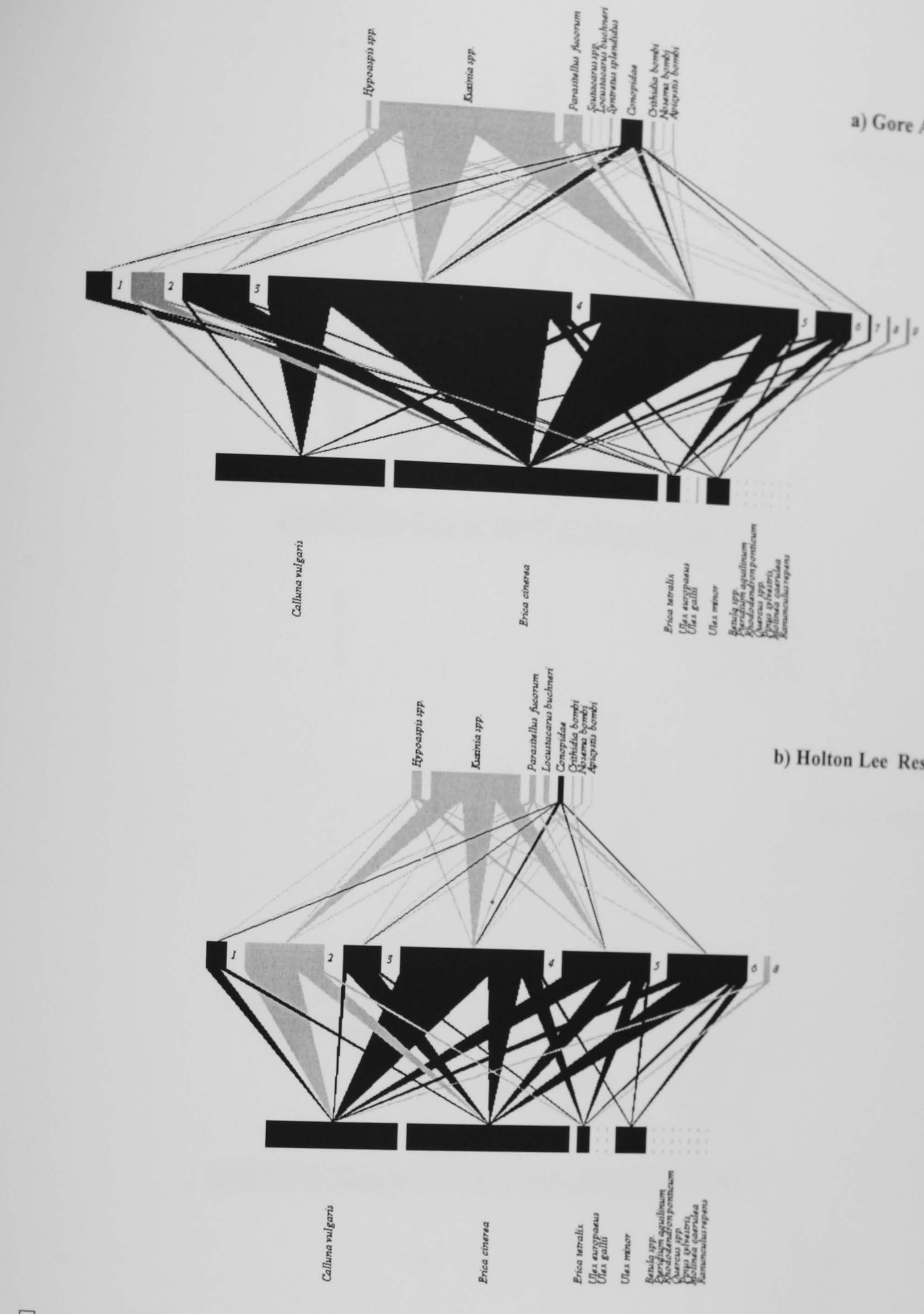
Appendix B



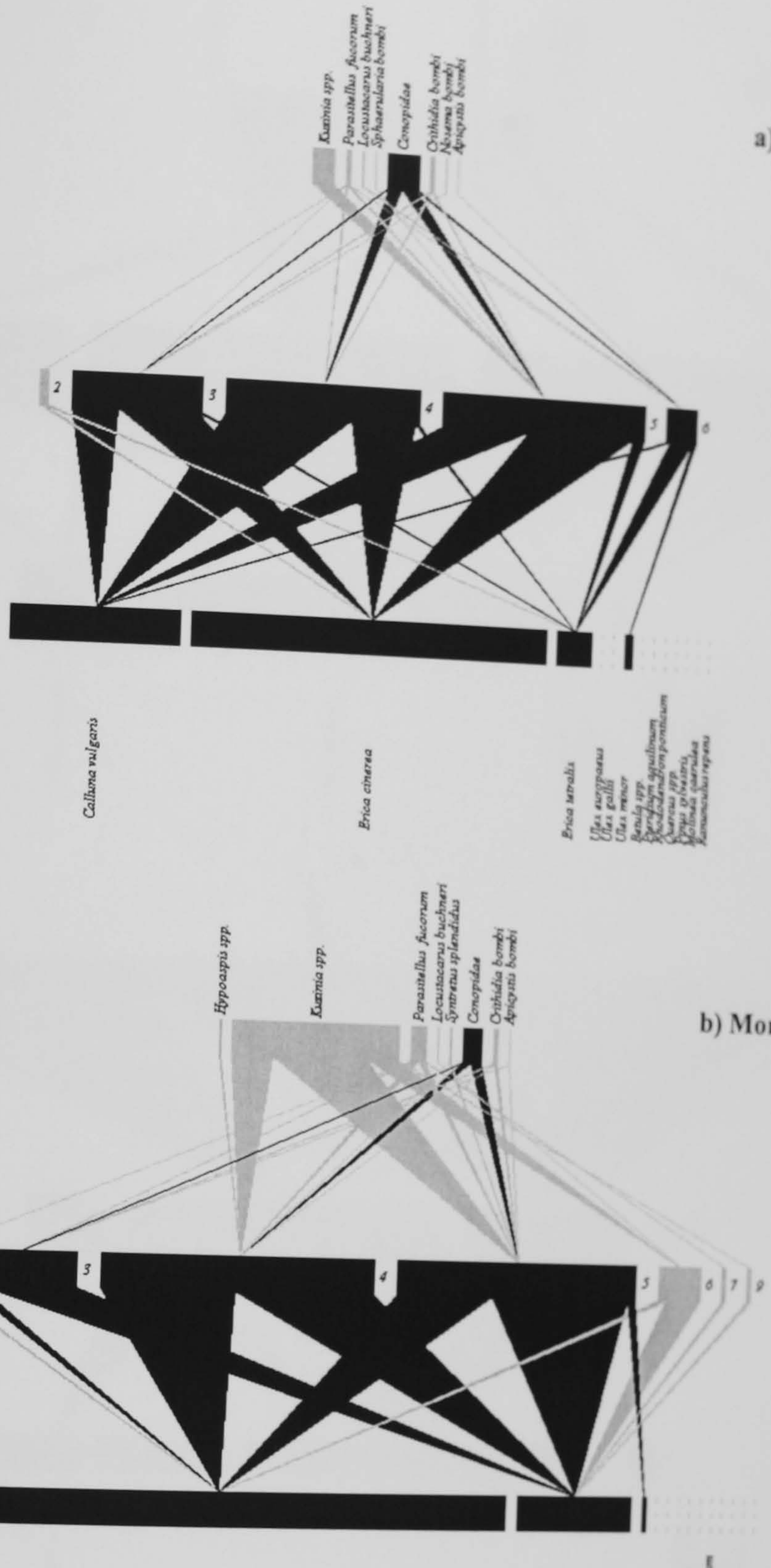
B.1 Quantitative interaction webs representing three trophic levels for a) ancient and b) restored heathland sites at Arne (Pair 1). The bottom row of rectangular bars represents the flower species, the middle and top row represent the bumblebee species and their natural enemies respectively. Each rectangular bar in a row represents a single species and their relative abundance is proportional to bar width. Bumblebee species labelled are: 1) *B. humilis,* 2) *B. jonellus,* 3) *B. lapidarius,* 4) *B. lucorum,* 5) *B. terrestris,* 6) *B. pascuorum and* 8) *B. pratorum*. The scale bar at the base of the figure represent: for flora, 100 floral units per m^2 of heathland; for bees, 10 individuals; and for their natural enemies, 100 individuals (with exception of the protozoan parasites *Crithidia, Nosema* and *Apicystis bombi,* where bar width is proportional to the number of hosts infected, as abundance could not be recorded). The solid triangles that connect trophic levels represent the interactions linking species; the size of the triangle being proportional to the frequency of the interaction. Both webs are drawn to the same scale and the conopid network is highlighted in black.



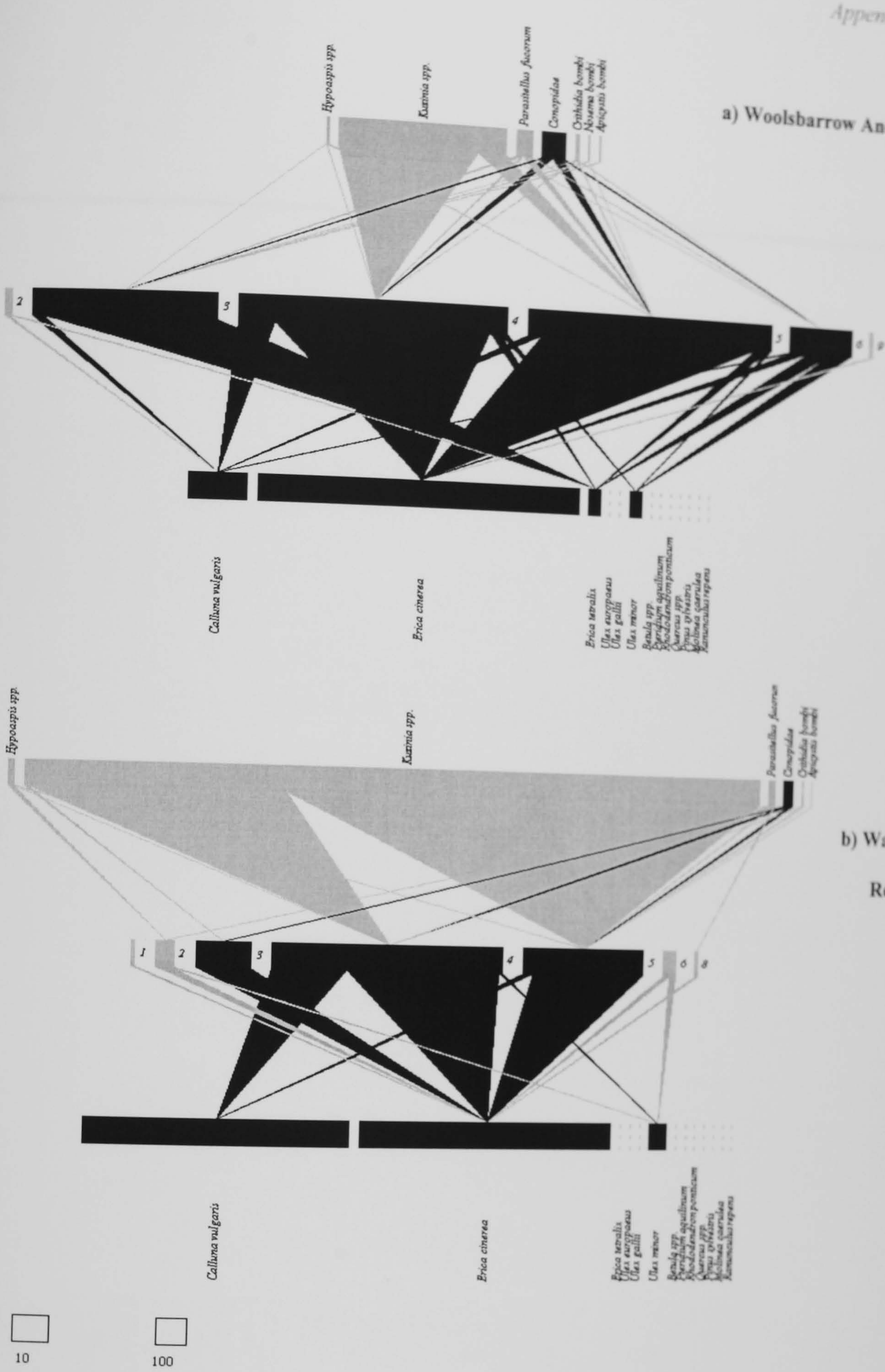
B.2 Quantitative interaction webs representing three trophic levels for a) ancient and b) restored heathland sites at Hyde (Pair 2). The bottom row of rectangular bars represents the flower species, the middle and top row represent the bumblebee species and their natural enemies respectively. Each rectangular bar in a row represents a single species and their relative abundance is proportional to bar width. Bumblebee species labelled are: 1) *B. humilis,* 2) *B. jonellus,* 3) *B. lapidarius,* 4) *B. lucorum,* 5) *B. terrestris,* 6) *B. pascuorum,* 8) *B. pratorum* and 9) *B. vestalis*. The scale bar at the base of the figure represent: for flora, 100 floral units per m^2 of heathland; for bees, 10 individuals; and for their natural enemies, 100 individuals (with exception of the protozoan parasites *Crithidia, Nosema* and *Apicystis bombi,* where bar width is proportional to the number of hosts infected, as abundance could not be recorded). The solid triangles that connect trophic levels represent the interactions linking species; the size of the triangle being proportional to the frequency of the interaction. Both webs are drawn to the same scale and the conopid network is highlighted in black.



B.3 Quantitative interaction webs representing three trophic levels for a) Gore ancient and b) Holton Lee restored heathland sites (Pair 3). The bottom row of rectangular bars represents the flower species, the middle and top row represent the bumblebee species and their natural enemies respectively. Each rectangular bar in a row represents a single species and their relative abundance is proportional to bar width. Bumblebee species labelled are: 1) *B. humilis,* 2) *B. jonellus,* 3) *B. lapidarius,* 4) *B. lucorum,* 5) *B. terrestris,* 6) *B. pascuorum,* 7) *B. hortorum,* 8) *B. pratorum* and 9) *B. vestalis*. The scale bar at the base of the figure represent: for flora, 100 floral units per m^2 of heathland; for bees, 10 individuals; and for their natural enemies, 100 individuals (with exception of the protozoan parasites *Crithidia, Nosema* and *Apicystis bombi,* where bar width is proportional to the number of hosts infected, as abundance could not be recorded). The solid triangles that connect trophic levels represent the interactions linking species; the size of the triangle being proportional to the frequency of the interaction. Both webs are drawn to the same scale and the conopid network is highlighted in black.



B.4 Quantitative interaction webs representing three trophic levels for a) ancient and b) restored heathland sites at Morden Bog (Pair 4). The bottom row of rectangular bars represents the flower species, the middle and top row represent the bumblebee species and their natural enemies respectively. Each rectangular bar in a row represents a single species and their relative abundance is proportional to bar width. Bumblebee species labelled are: 2) *B. jonellus,* 3) *B. lapidarius,* 4) *B. lucorum,* 5) *B. terrestris,* 6) *B. pascuorum,* 7) *B. hortorum* and 9) *B. vestalis*. The scale bar at the base of the figure represent: for flora, 100 floral units per m^2 of heathland; for bees, 10 individuals; and for their natural enemies, 100 individuals (with exception of the protozoan parasites *Crithidia, Nosema* and *Apicystis bombi,* where bar width is proportional to the number of hosts infected, as abundance could not be recorded). The solid triangles that connect trophic levels represent the interactions linking species; the size of the triangle being proportional to the frequency of the interaction. Both webs are drawn to the same scale and the conopid network is highlighted in black.



B. 5 Quantitative interaction webs representing three trophic levels for a) Woolsbarrow Fort ancient and b) Wareham Forest restored heathland sites (Pair 5). The bottom row of rectangular bars represents the flower species, the middle and top row represent the bumblebee species and their natural enemies respectively. Each rectangular bar in a row represents a single species and their relative abundance is proportional to bar width. Bumblebee species labelled are: 1) *B. humilis,* 2) *B. jonellus,* 3) *B. lapidarius,* 4) *B. lucorum,* 5) *B. terrestris,* 6) *B. pascuorum,* 8) *B. pratorum* and 9) *B. vestalis*. The scale bar at the base of the figure represent: for flora, 100 floral units per m^2 of heathland; for bees, 10 individuals; and for their natural enemies, 100 individuals (with exception of the protozoan parasites *Crithidia, Nosema* and *Apicystis bombi,* where bar width is proportional to the number of hosts infected, as abundance could not be recorded). The solid triangles that connect trophic levels represent the interactions linking species; the size of the triangle being proportional to the frequency of the interaction. Both webs are drawn to the same scale and the conopid network is highlighted in black.

Appendix C

C.1 Presence/absence table of the plant species sampled in the summer of 2006, Colorado Rocky Mountains, USA. Plots sampled were from 5 replicate valley sites between Crested Butte and Gothic: Gothic (GT), Lower Loop (LL), Moon Ridge (MR), Slate River (SR) and Washington Gulch (WG). 3 plot treatments were studied per site: *C. arvense* present and *L. vulgaris* absent (Ca), *L. vulgaris* present and *C. arvense* absent (Lv), and mixed plots where both species co-occurred in relatively equal densities (Mixed).

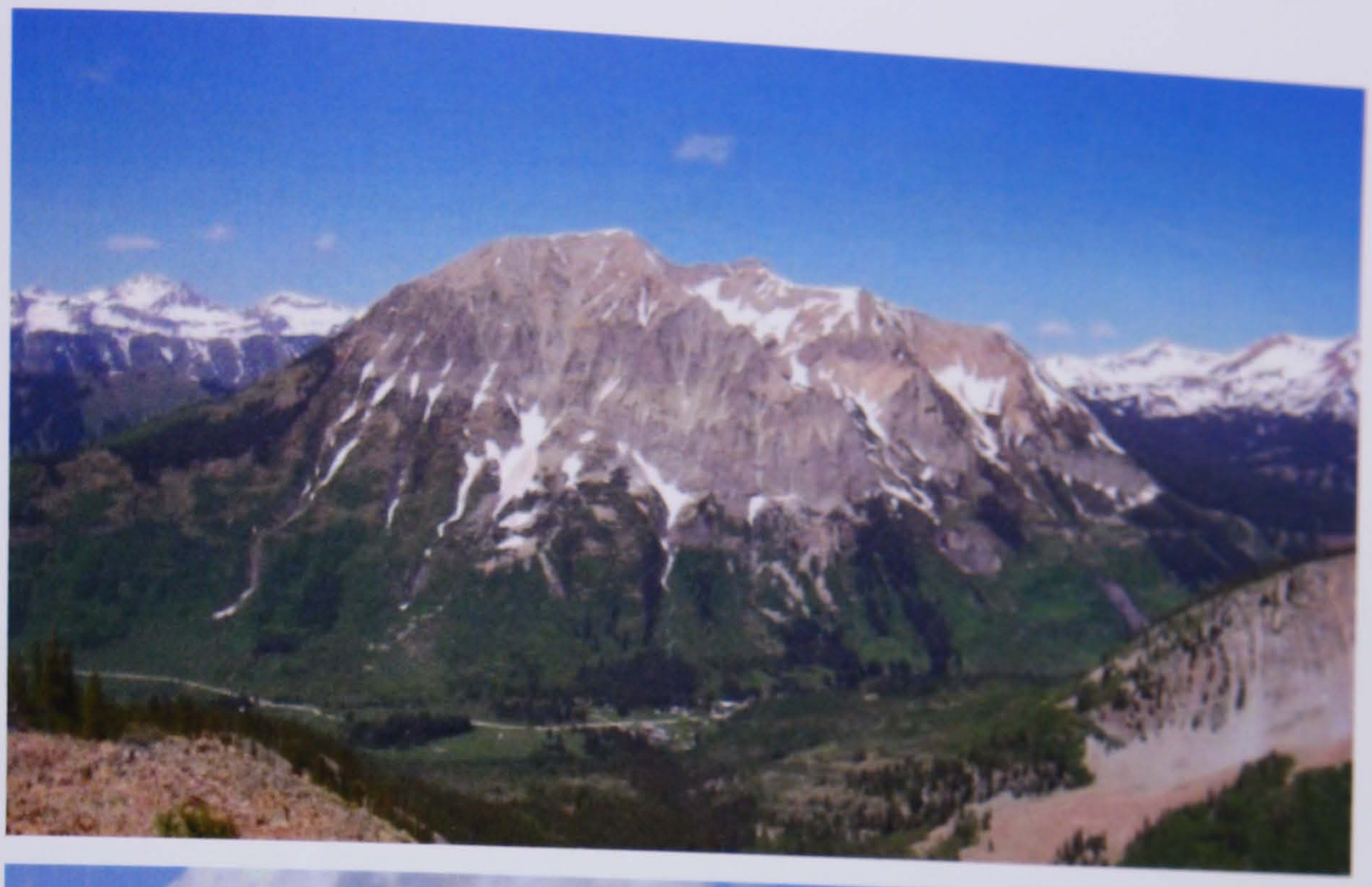
		Date sampled per site (DD/MM)														
		21/07 - 04/09			19/07 - 03/09			13/08 - 02/09			20/07 - 12/08			18/07 - 12/08		
		GT			LL			MR			SR			WG		
Plant species	Common name	Ca	Lv	Mixed	Ca	Lv	Mixed	Ca	Lv	Mixed	Ca	Lv	Mixed	Ca	Lv	Mixed
Achillea millefolium	yarrow	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Aconitum columbianum	columbian monkswood		•				•						•			
Agastache urticifolia	horsemint				•	•	•									
Antennaria alpina	alpine pussytoes					•										
Arnica mollis	hairy arnica					•										
Artemisia tridentata	sagebrush							•			•			•	•	•
Aster foliaceus	leafy aster	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Calochortus apiculatus	mariposa lilly														•	
Campanula parryi	parry's harebell					•	•		•			•	•			
Campanula rotundifolia	common harebell			•	•		•	•						•	•	•
Castilleja miniata	scarlet paintbrush				•						•					
Castilleja occidentalis	western yellow paintbrush								•				•			
Chicorium intybus	wild chicory								•							
Circium arvense	canada / creeping thistle	•		•		•	•		•	•					•	•
Circium hookerianum	hooker's thistle		•													
Collomia linearis	narrow-leaved collomia		•													
Delphinium glaucum	tall larkspur	•	•	•												
Descurainia sophia	tansymustard	•	•								•					
Erigeon glabellus	smooth fleabane					•										
Erigeron peregrinus	subalpine fleabane							•								
Erigeron sp. 1						•										
Erigeron sp. 2						•										
Erigonum umbellatum	sulphur buckwheat										•			•	•	
Galium boreale	northern bedstraw	•		•	•		•						•		•	
Gentiana calycosa	mountain bog gentian			•				•								
Geranium bicknellii	bicknell's geranium				•			•	•	•				•	•	•
Hackelia floribunda	many-flowered stickseed	•	•	•												•
Helianthella quinquenervis	sunflower	•														
Heliomeris multiflora	goldeneye/sunspots	•	•	•	•	•	•		•		•	•		•		•
Heracleum maximum	cow-parsnip	•	•	•												
Heterotheca villosa	golden-aster		•	•	•	•	•	•				•				
Hymenoxys hoopsii		•	•	•	•	•	•							•	•	•
Ipomopsis aggregata	scarlet gilia / skyrocket	•		•	•			•	•		•	•		•		•
Leucanthemum vulgare	ox-eye daisy					•						•	•			
Ligusticum porteri	loveroot						•						•			
Linaria vulgaris	toadflax / butter & eggs	•	•	•	•	•		•	•		•	•		•	•	
Linum lewisii	wild blue flax	•	•	•					•					•		
Lupinus lepidus	cushion lupin							•								
lupinus sericeus	silky lupine							•	•							
Mertensia paniculata	tall bluebells	•		•												
Penstemon albertinus	Alberta pentstemon						•									
Penstemon eriantherus	fuzzy-tongued penstemon		•		•						•	•				
Pentaphylloides floribunda	shrubby cinquefoil				•	•	•		•	•		•	•	•	•	•
Ranunculus acris	meadow buttercup	•	•	•	•	•	•			•			•			•
Rumex sp.	dock		•													
Senecio bigelovii	nodding ragwort	•	•	•			•		•	•						
Silene latifolia/alba	white campion		•				•		•	•						
Solidago canadensis	canada goldenrod								•				•	•	•	•
Solidago sp. 1	goldenrod sp. 1				•	•	•	•	•		•				•	
Solidago sp. 2	goldenrod sp. 2							•						•	•	•
Taraxacum officinale	dandelion	•	•	•	•	•	•		•							•
Tragopogon dubius	yellow salsify			•												
Tragopogon pratensis	meadow salsify						•	•	•			•	•			
Trifolium pratense	clover	•	•		•	•										
Urtica dioica	stinging nettle		•													
Veratrum viride	green false-hellebore		•							•	•					
Vicia americana	wild vetch	•		•	•											

C.2 Presence/absence table of the insect morphospecies sampled in the summer of 2006, Colorado Rocky Mountains, USA. Plots sampled were from 5 replicate valley sites between Crested Butte and Gothic: Gothic (GT), Lower Loop (LL), Moon Ridge (MR), Slate River (SR) and Washington Gulch (WG). 3 plot treatments were studied per site: *C. arvense* present and *L. vulgaris* absent (Ca), *L. vulgaris* present and *C. arvense* absent (Lv), and mixed plots where both species co-occurred in relatively equal densities (Mixed). Voucher specimens are retained at the University of Bristol.

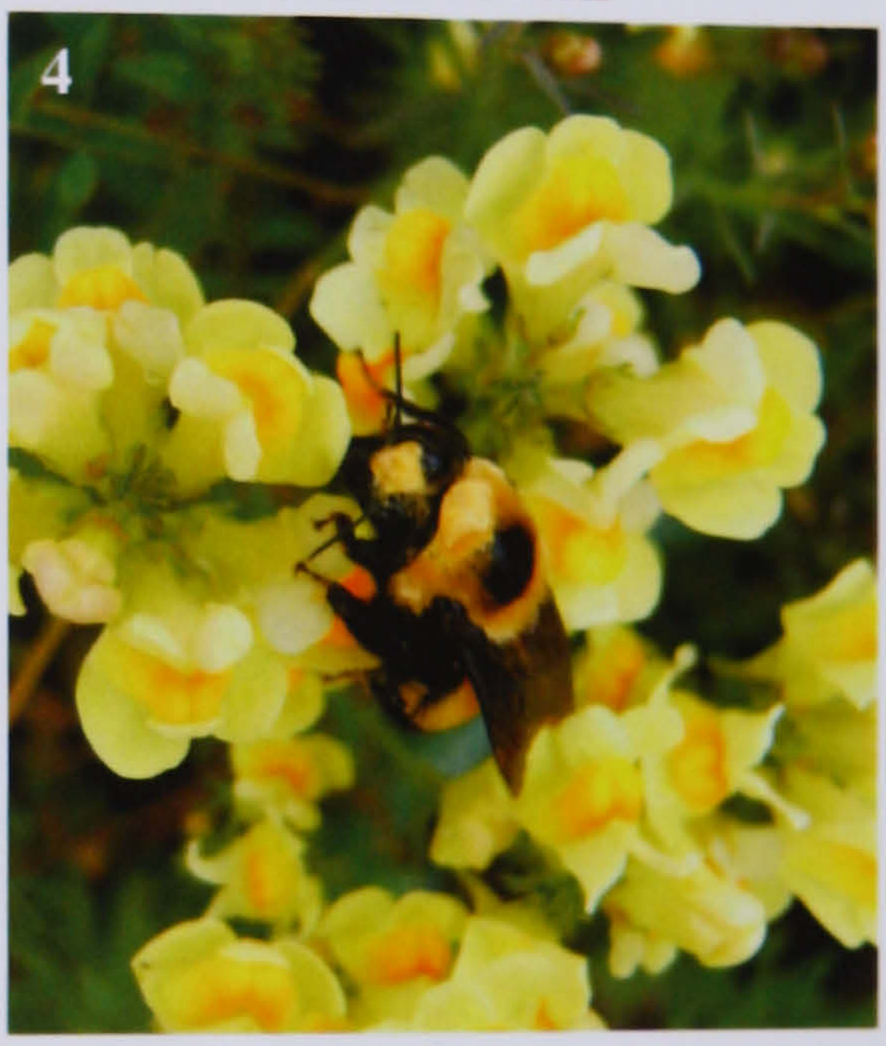
		18/07 - 05/09		5/07 - 03/09		14/08 - 03/09		20/07 - 07/08		17/07 - 10/08	
		GT		LL		MR		SR		WG	
Insect morphospecies	**Order**	on Lv	on Ca	on Lv	on Ca	on Lv	on Ca	on Lv	on Ca	on Lv	on Ca
Bombus (psithyrus) insularis	Hymenoptera	•		•							
Bombus appositus	Hymenoptera	•	•	•	•	•	•	•		•	
Bombus bifarius	Hymenoptera	•	•	•	•	•	•	•		•	
Bombus californicus	Hymenoptera	•		•		•	•	•			
Bombus flavifrons	Hymenoptera		•		•						
Bombus frigidus	Hymenoptera	•								•	
Bombus nevadensis	Hymenoptera	•		•		•	•	•		•	
Bombus occidentalis	Hymenoptera	•	•	•	•	•	•	•			
Calliphoridae	Diptera	•	•		•		•				
Coleoptera sp. B	Coleoptera				•						
Coleoptera sp. C	Coleoptera										•
Coleoptera sp. D	Coleoptera		•								
Diptera sp. A	Diptera		•	•	•		•				
Diptera sp. B	Diptera			•							
Diptera sp. C	Diptera									•	
Diptera sp. D	Diptera		•	•	•		•		•		•
Diptera sp. E	Diptera		•				•				
Diptera sp. F	Diptera		•								
Diptera sp. G	Diptera		•		•		•				
Diptera sp. H	Diptera		•		•		•				
Diptera sp. I	Diptera		•		•		•				
Diptera sp. J	Diptera		•				•				
Diptera sp. K	Diptera						•				
Diptera sp. M	Diptera		•								
Diptera sp. Q	Diptera		•		•						
Diptera sp. R	Diptera		•								
Diptera sp. S	Diptera		•								
Hylaeus lineata (sphinx moth)	Lepidoptera			•		•	•				
Hymenoptera sp. A	Hymenoptera	•	•	•	•		•		•		•
Hymenoptera sp. B	Hymenoptera		•								
Hymenoptera sp. C	Hymenoptera		•		•						
Hymenoptera sp. D	Hymenoptera		•								
Ladybird A	Coleoptera				•						
Ladybird B	Coleoptera				•					•	
Lepidoptera sp. A	Lepidoptera			•				•			
Lepidoptera sp. B	Lepidoptera		•								
Lepidoptera sp. C	Lepidoptera		•	•	•						
Lepidoptera sp. D	Lepidoptera		•				•				
Lepidoptera sp. E	Lepidoptera	•	•								
Lepidoptera sp. F	Lepidoptera		•								
Lepidoptera sp. G	Lepidoptera		•								
Lepidoptera sp. H	Lepidoptera		•								
Lepidoptera sp. I	Lepidoptera	•	•		•	•	•				
Lepidoptera sp. J	Lepidoptera				•		•	•		•	
Nitidulidae	Coleoptera										

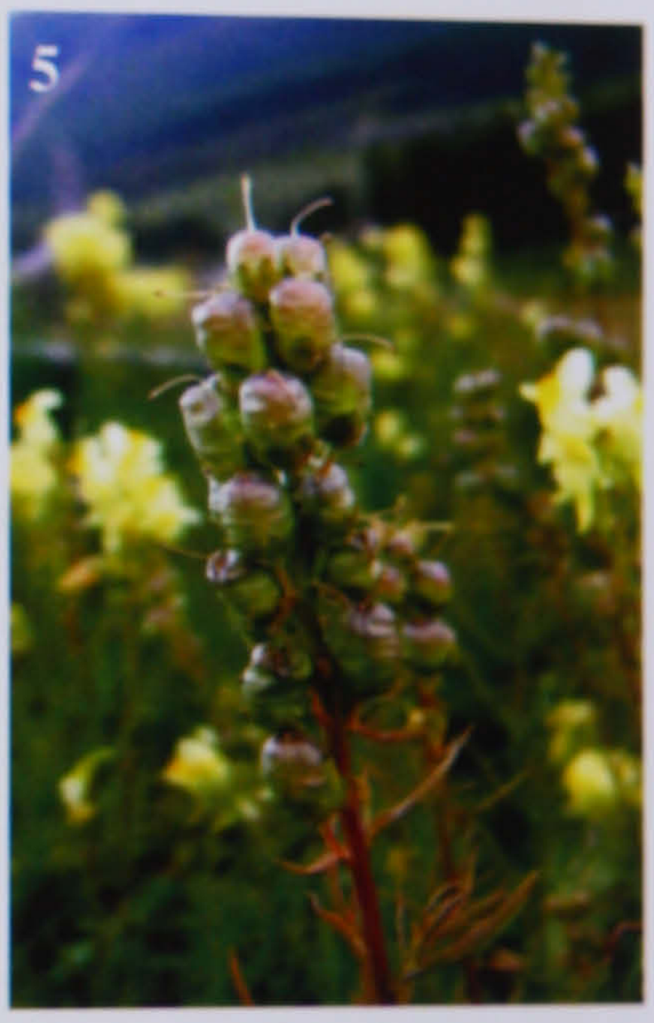
C.2 continued.

Insect morphospecies	Order	18/07 - 05/09		5/07 - 03/09		14/08 - 03/09		20/07 - 07/08		17/07 - 10/08	
		GT		LL		MR		SR		WG	
		on Lv	on Ca	on Lv	on Ca	on Lv	on Ca	on Lv	on Ca	on Lv	on Ca
Selasphorus platycercus (Broad-tailed hummingbird)	Trochilidae	•		•		•	•	•			
Solitary bee A	Hymenoptera	•		•				•		•	
Solitary bee B	Hymenoptera			•	•						
Solitary bee C	Hymenoptera	•	•	•	•			•			
Solitary bee D	Hymenoptera		•		•			•			
Solitary bee E	Hymenoptera			•							
Solitary bee F	Hymenoptera		•					•			
Solitary bee G	Hymenoptera		•		•						
Solitary bee H	Hymenoptera	•								•	
Solitary bee I	Hymenoptera				•		•				
Solitary bee K	Hymenoptera				•						
Solitary bee L	Hymenoptera		•								
Solitary bee M	Hymenoptera		•		•		•				
Solitary bee N	Hymenoptera		•		•						
Solitary bee O	Hymenoptera		•								
Solitary bee P	Hymenoptera		•		•						
Solitary bee Q	Hymenoptera		•								
Solitary bee R	Hymenoptera		•								
Syrphidae sp. A	Diptera	•	•	•	•		•	•		•	
Syrphidae sp. B	Diptera	•	•	•	•		•	•		•	
Syrphidae sp. C	Diptera		•				•				
Syrphidae sp. D	Diptera		•		•						
Syrphidae sp. E	Diptera		•		•		•				•
Syrphidae sp. F	Diptera		•		•						
Syrphidae sp. G	Diptera		•		•		•				
Syrphidae sp. H	Diptera		•		•						
Syrphidae sp. I	Diptera		•		•		•				
Syrphidae sp. J	Diptera		•		•		•				
Syrphidae sp. K	Diptera						•				
Syrphidae sp. L	Diptera		•		•		•				
Syrphidae sp. M	Diptera		•				•				
Syrphidae sp. N	Diptera		•				•				
Syrphidae sp. O	Diptera				•						
Syrphidae sp. P	Diptera						•				
Syrphidae sp. Q	Diptera				•		•				
Syrphidae sp. R	Diptera				•						
Volucella bombylans	Diptera	•	•		•		•				
Wasp A	Hymenoptera	•	•	•	•		•	•	•		•
Wasp A2	Hymenoptera	•	•	•	•		•				
Wasp B	Hymenoptera	•	•	•	•		•				
Wasp C	Hymenoptera	•	•		•						
Wasp D	Hymenoptera		•		•						
Wasp E	Hymenoptera		•	•		•					
Wasp F	Hymenoptera		•								
Wasp G	Hymenoptera		•		•						
Wasp H	Hymenoptera				•						
Wasp I	Hymenoptera						•				
Wasp J	Hymenoptera				•						
Wasp K	Hymenoptera		•								
Wasp L	Hymenoptera		•								
Wasp M	Hymenoptera		•								



Alpine habitat around the Rocky Mountains Biological Laboratory, at Gothic, Colorado (106°59'15" N; 38°57'30" W; 2900m).





Plates 1 & 2) Invasive extent of *Linaria vulgaris* (Common Toadflax) at RMBL; 3) *L. vulgaris* in flower; 4) *B. nevadensis* foraging on *L. vulgaris;* 5) *L. vulgaris* in seed, 6) *L. vulgaris* seeds.

Plates in clockwise direction: 1 & 2) Invasive extent of *Cirsium arvense* (Creeping thistle) at RMBL; 3) seeds / achenes of *C. arvense*, 4) feathery pappus of *C. arvense*.